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DECOMPOSITION AND REGENERATION OF NITROGENOUS ORGANIC MATTER IN SEA WATER [1]

III. Influence of Temperature and Source and Condition of Water

THEODOR VON BRAND AND NORRIS W. RAKESTRAW

(From the Woods Hole Oceanographic Institution, Woods Hole, Mass.)

The first two papers in this series (1937, 1939) have shown that the decomposition of suspended organic matter is accompanied by the successive formation of ammonia, nitrite and nitrate in the sea water medium in distinctly recognizable stages; that fresh diatoms can grow in the water at any stage in the cycle; and that successive cycles of decomposition and regeneration can be carried out in the same water, without the addition of new organic substrate. Since the analogous processes in the sea can scarcely be expected to take place in such simple, successive fashion, it is important to know how the decomposition cycle is influenced in rate or character by a number of variable factors. Among these are temperature, source of the sea water, and the presence or absence of such micro-organisms as can be controlled by the routine technic of sterilization. The present paper describes an experimental study of these factors.

Earlier work had shown that the nature of the decomposition cycle varied somewhat with the source of the water, being generally more rapid in water from the harbor than in that from offshore. To pursue this point further three samples of water were taken from a deep-sea station (25°–32′ N.; 53°–45′ W.): one from the surface, one from the level of the oxygen minimum (800 meters), and one from 1200 meters. Each of these samples was divided into two parts, one of which was "sterilized" by suction filtration through a No. 5 sintered-glass funnel. There were previous indications that this treatment was adequate to remove bacteria.

To each of the six portions of sea water (about two liters each) were added equal amounts of washed *Nitzschia Closterium*, which, as in

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previous experiments, served as a convenient source of organic matter. The suspensions were placed in the dark, at laboratory temperature, and periodically analyzed for particulate nitrogen, ammonia, nitrite and nitrate, in the manner already described. The results are shown in Fig. 1.

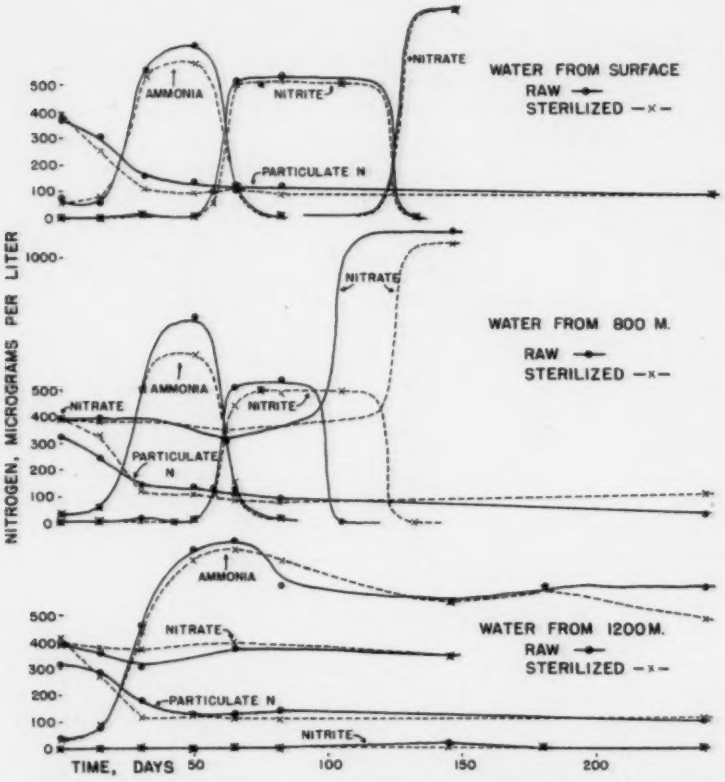

FIG. 1. The disappearance of particulate nitrogen and the simultaneous changes in ammonia, nitrite and nitrate. Fresh cultures of *Nitzschia Closterium* suspended in sea water from different depths, part of which has been sterilized by filtration.

There was no difference in the behavior of those samples which had been sterilized by filtration and those which had not. The decomposition proceeded in the normal manner in the surface water and in that from the oxygen minimum layer. But in the deep water (1200 m.)

there was no oxidation of ammonia during the time of the experiment (250 days). The nature of this inhibiting effect is yet to be determined. That it is not due simply to the absence of bacteria seems evident from the fact that such oxidation did proceed in the other samples, whether or not the water had been sterilized. This would indicate that the necessary organisms were carried by the diatoms. But since the same diatom culture was used in the deep water, the failure of oxidation to take place is evidently due to some other, unexplained factor. It should be pointed out that ammonia and nitrite are seldom found below 200 or 300 meters in the sea.

Since the oxidizing organisms seemed to be carried by the diatoms rather than in the water itself, another series of experiments was carried out in which harbor water was used as the suspension medium, part of which was sterilized by filtration, as before. To portions of the sterile and the raw water washed diatoms were added, as in the last series. To other similar portions of the water (sterile and raw) were added equal amounts of a diatom suspension which had been boiled for two minutes to sterilize it. Precautions were taken to insure the addition of equal amounts of organic matter to all four of the cultures, after which they were placed in the dark and sampled periodically, according to the usual routine. The results are shown in Fig. 2.

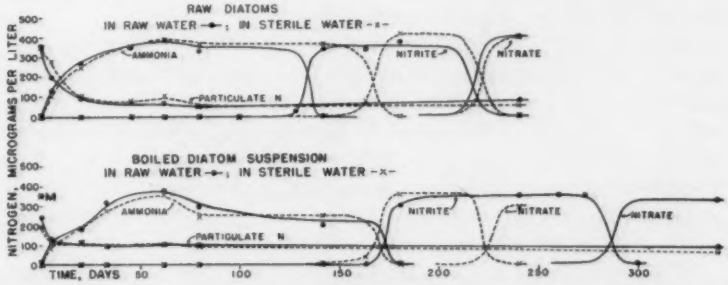

FIG. 2. The disappearance of particulate nitrogen and the simultaneous changes in ammonia, nitrite and nitrate, in harbor water, one portion sterilized by filtration. Upper curves: fresh cultures of *Nitzschia Closterium*. Lower curves: *Nitzschia* cultures boiled to sterilize. The point marked *M indicates the amount of particulate nitrogen in the *Nitzschia* culture before boiling.

The boiling of the diatom suspension quite evidently throws some of the organic matter into solution, for, although equal quantities of diatoms were used, the first determination of particulate nitrogen is much lower in the cultures to which the boiled suspensions were added than in those containing the same amount of raw diatom material. The

boiling of the diatoms also resulted in a somewhat slower rate of ammonia formation and a prolongation of the ammonia stage of the cycle.

The fact that the decomposition cycles were so nearly alike in the sterilized and the raw sea water, and that oxidation of ammonia to nitrite and nitrate took place in all cases, raises the serious question whether the filtration had actually sterilized the sea water, or whether the cultures had been contaminated with nitrite-forming organisms from the air. It is difficult to explain the formation of nitrite and nitrate except upon one of these two assumptions and we are not as yet able to resolve this difficulty. The culture methods for the identification of nitrite-forming bacteria are so unsatisfactory that we did not attempt to use them upon our material. It is almost impossible to use aseptic technic in sampling the cultures, although the chance of contamination can be diminished by pouring rather than pipetting samples.

In the effort to get further information on this point, four supposedly bacteria-free cultures of *Nitzschia* were obtained. One of these was analyzed immediately and found to be free of ammonia, nitrite and nitrate. The others were placed in the dark and analyzed later. After 145 days one culture contained 500 γ of ammonia nitrogen and 10 γ of nitrite nitrogen per liter. After 300 days the second contained 870 γ of ammonia nitrogen and 35 γ of nitrite. A large amount of suspended material still remained in both cases, but it was evident that considerable decomposition had taken place.

The last culture was opened and large numbers of bacteria were found in the remaining suspended matter. Even with great care we had not succeeded in preparing and preserving sterile cultures. Nevertheless, it seems worthwhile to note that bacteria developed only very slowly and that an almost negligible amount of ammonia had been oxidized to nitrite in 300 days. Apparently it is easier to eliminate the organisms responsible for oxidation than those which may possibly take part in the formation of ammonia.

To determine the temperature coefficient of the decomposition process, three suspensions of washed cells of *Nitzschia* were prepared in the usual manner. These were then placed in the dark at constant temperatures: one at 15° C., one at 7–9° C., and one at 1–2° C. By a stroke of good luck, when the first complete analysis was made, after 280 days, the one at the lowest temperature (No. 28) was in the ammonia stage, the one at the middle temperature (No. 29) was in the nitrite stage, and the one at the highest temperature (No. 30) had completed the cycle and all the nitrogen was in the form of nitrate. The complete results are given in Table I.

Unfortunately, No. 29 was lost at about the time when the nitrite had disappeared, but the cycle was so nearly completed at the time of the last preceding analysis that a sufficiently accurate estimate of the total elapsed time could be made. At the date of writing, after 635 days, No. 28 had not gone beyond the ammonia stage.

TABLE I

Series Nos. 28, 29 and 30. Sea water containing fresh cultures of *Nitzschia Closterium* kept in the dark at different temperatures as shown. Micrograms of nitrogen per liter.

	Date	Particulate	Ammonia	Nitrite	Nitrate
No. 28	9–14–38	470	10	0	10
Temperature	10– 3–38	260			
1 to 2° C.	1– 6–39	244			
	4–17–39	98			
	6–22–39	134	385	0	15
	8–30–39	64			
	2–26–40	24	310	0	10
	6–11–40	24	385	0	5
No. 29	9–14–38	470	10	0	10
Temperature	10– 3–38	129			
7 to 9° C.	1– 6–39	75			
	4–17–39	51			
	6–22–39	67	21	330	10
	8–30–39	41	20	320	
	9– 7–39			200	
	2–26–40	25	0	45	
No. 30	9–14–38	483	5	0	10
Temperature	10– 3–38	38			
15° C.	1– 6–39	49			
	4– 7–39	47			
	6–22–39	57	18	0	375
	8–30–39	28	25	0	400
	2–26–40	40	0	0	400
	6–11–40	18	0	0	400

The complete cycle in No. 30 (15°) took less than 280 days, and in No. 29 (7–9°) about 550 days. A temperature difference of 6 to 8° C. therefore more than doubled the velocity of the over-all process. Since more than one reaction mechanism is involved in the cycle, the relation is probably not a simple one, and it is indeed possible that a certain minimum temperature is necessary for the oxidation of ammonia.

On September 2, 1939, portions of cultures Nos. 28, 29 and 30 were inoculated with fresh diatoms and placed in the light. At this time the maximum amounts of ammonia, nitrite and nitrate, respectively, were

in these three cultures. Two weeks later there was no ammonia, nitrite or nitrate in any of the three, and an abundant diatom growth was observed. This confirms definitely our earlier observation that new diatoms can grow in the medium at any stage in the cycle of decomposition.

Summary

The source of the water is important in determining the nature of the decomposition cycle. Oxidation of ammonia to nitrite is retarded in water from the deep sea (1200 m.). Inconclusive results were obtained from efforts to sterilize both the water and the original organic matter, but it is evidently easier to eliminate organisms responsible for the oxidation processes than those which may participate in the formation of ammonia. The speed of the whole decomposition cycle was more than doubled by an increase of 6° or 8° in temperature. Growth of diatoms is possible at any stage in the cycle of decomposition.

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THE CHROMATOPHORE SYSTEM IN THE CATFISH AMEIURUS

G. H. PARKER

(From the Biological Laboratories, Harvard University)

Introduction

The color changes in the catfish, *Ameiurus nebulosus*, have attracted general attention for at least two decades, but they have been studied in detail only during the last five or six years. As receptors for such changes the eyes in this fish have been recognized by all workers. In 1917 Van Heusen showed that the innervation of the catfish skin was photosensitive and some two years ago Wykes (1938) pointed out that this sensitivity may play a part in the color changes in this fish. The pineal organ has also been suspected of influencing the chromatic activities in *Ameiurus* and the direct stimulation of chromatophores by light has been invoked by some workers to this end.

The chromatophores in *Ameiurus* appear to be all melanophores, micromelanophores in the epidermis and macromelanophores directly under the derma. Of these the macromelanophores are much the more convenient for study. The melanophores in *Ameiurus* appear to be activated through three sets of neurohumors, intermedin from the pituitary gland, acetylcholine from the dispersing autonomic nerve-fibers and adrenaline from the concentrating fibers. The first two neurohumors disperse the melanophore pigment and thereby darken the fish; the third concentrates it and thus induces blanching (Parker, 1940*a*). The object of this paper is to discuss the systematic relations of catfish melanophores, their neurohumors, and the receptors concerned with their activation.

Specific Responses of Melanophores

The melanophores of *Ameiurus* respond very differently depending upon the particular conditions of the fish: presence or absence of eyes, of pituitary gland, whether the fish is in darkness or in light, and the nature of the background white or black. Many of these types of response have been recorded already by students of this subject some of whom have partly tabulated them (Abramowitz, 1936; Osborn, 1938*b*).

Table I of the present paper summarizes my own observations on the tint of the tail of *Ameiurus,* which is the same as that of the body generally, as well as on the tint of newly cut caudal bands under different conditions. These conditions, of which sixteen are recorded, are indicated on the left-hand side of Table I.

TABLE I

Tints of the tails and of the newly cut caudal bands of the catfish *Ameiurus* under various conditions: with or without eyes, with or without pituitary glands, in light on white or black backgrounds, and in darkness as pale fishes or dark ones. The scale of tints in relation to the average diameters in microns of the macromelanophores is approximately as follows: pale, 45; palish, 60; intermediate 70; darkish, 95; dark, 138; coal-black, 143.

Conditions of fishes		Tints	
		Tail	New band
Eyed			
With pituitary gland			
In light			
White background	1	Pale	Darkish
Black background	2	Dark	Coal-black
In darkness			
Pale fish	3	Pale	Darkish
Dark fish	4	Dark	Coal-black
Without pituitary gland			
In light			
White background	5	Palish	Darkish
Black background	6	Intermediate	Darkish
In darkness			
Pale fish	7	Palish	Darkish
Intermediate fish	8	Intermediate	Darkish
Eyeless			
With pituitary gland			
In light			
White background	9	Coal-black	Coal-black
Black background	10	Coal-black	Coal-black
In darkness			
Pale fish	11	Pale	Darkish
Dark fish	12	Dark	Coal-black
Without pituitary gland			
In light			
White background	13	Intermediate	Darkish
Black background	14	Intermediate	Darkish
In darkness			
Pale fish	15	Palish	Darkish
Intermediate fish	16	Intermediate	Darkish

The diameters of the pigmented areas of the macromelanophores in *Ameiurus* are about 40 microns in the concentrated state and 145 microns in the dispersed one and of the micromelanophores about 12 and 90 microns respectively. Wykes (1938) and Osborn (1938*b*) in their

studies of the catfish color changes have measured the diameters of considerable numbers of melanophores under different conditions and expressed their results in averages. My own procedure has been somewhat different. I have preferred to use adjectives as descriptive of the states of the color cells instead of cell measurements and I have distinguished in the scale of changes six steps from extreme dispersion of pigment to its extreme concentration (Table I). These steps have been judged in any given fish by the eye in comparison with the tints of other fishes kept as standards. This can be illustrated by the most difficult steps to distinguish that between dark and coal-black.

To carry out this separation standards were made in the following way. Two catfishes closely matched in color were kept in an illuminated, black-walled aquarium for several days till they had come to a uniform deep tint, dark. The eyes of one of these fishes were then removed whereupon the fish, still illuminated, quickly deepened in tint to what has been called coal-black. In the illuminated, black-walled aquarium these two fishes, one dark and the other coal-black, continued to maintain with great persistence these two tints. They could then be used as standards for estimating the colors of other experimental fishes. By appropriate steps additional fishes were standardized for other points in the scale and the fishes thus prepared were used in judging the color of fishes under test. In my opinion this method is a more delicate and certain one for determining tints than that of measuring macromelanophores.

Another feature in the measurement of melanophores should be mentioned. Catfishes of the lengths used in these studies, about 15 cm., are continually losing melanophores and forming new ones (Odiorne, 1937). Small macromelanophores are therefore presumably young ones and in process of increasing in size. As a means of comparison in the present study a considerable number of melanophores under a variety of conditions were measured, but in consequence of the way in which melanophores grow these measurements were made not on the total population of color cells but on the large and presumably mature cells. This selective process is reasonable, for what is of concern at this stage is not the diameters of melanophores in general, but the diameters of their pigment areas as seen in their dispersed and their concentrated states.

On several occasions during these studies full sets of melanophore measurements were carried through on all the conditions of catfishes noted in Table I. These consisted of micrometer eye-piece measurements on at least ten mature macromelanophores in each of three catfishes for all of the sixteen states given in the table. These measure-

ments afforded the basis for the rough estimates of the diameters of macromelanophores in relation to the tints of the fishes as stated in the heading for Table I. In general they are in line with the kind of measurements made by Wykes and by Osborn. Of these steps in color changes the one from dark to coal-black has long been known and recognized as of considerable importance. Yet its difference in terms of melanophore diameters, about five microns, is scarcely within measurable limits though it is easily noted by direct inspection. Hence, as already stated, inspection by eye and the use of standard fishes may be a more effective way of discovering color differences than measuring melanophores.

No detailed comments as to the contents of Table I will be made here, but the data in this table will be referred to frequently in the course of the following discussion. A few general remarks, however, may not be inappropriate. The fully pale fishes are those with eyes, pituitary glands, in bright light and on a white background (Table I, 1). This high degree of paleness disappears with the loss of the pituitary gland (5). The coal-black fishes are eyeless, but with pituitary glands and in the light (9, 10). Newly cut bands are coal-black only when induced in fishes which have not been hypophysectomized (2, 4, 9, 10, 12). No hypophysectomized fish either in the tint of its tail or in that of its band ever reaches full paleness or coal-blackness (5 to 8 and 13 to 16). These limitations have already been pointed out by Osborn (1938*a*), Veil (1938), Wykes (1938), Enami (1939), and Parker (1940*a*). The states in darkness recorded in the table involve resting states of melanophores (Parker, 1939; 1940*b*) and hence fishes showing the two possible extremes of tint at the time of their introduction into darkness are given, pale or dark for those with pituitary glands (3, 4, 11, 12) and palish or intermediate for those without these glands (7, 8, 15, 16).

The Activation of Melanophores

The activation of the melanophores in *Ameiurus* has been attributed to the direct action of light on these color cells or by means of such receptors as the pineal organ, integumentary photoreceptors, and the eyes. The direct action of light on the melanophores of the catfish was suggested as early as 1934 by Parker. It was tested experimentally by Wykes (1938) who found that a narrow beam of light focused on the skin of a catfish by means of a lens was not followed by any chromatophoral response. I have also tested this matter experimentally. The tail of a catfish of intermediate tint was split longitudinally into halves by an incision running from the edge of the tail to its root and parallel

with its rays. A fish with a tail thus split was tied on its side to a black board and provided with a current of fresh water through its mouth and gills for respiration. The split tail was then pinned out on the board with one half of it freely exposed and the other half covered with a light-proof, metal chamber so fitted into the board and about the tail that the portion of the tail within it was entirely inaccessible to light. The whole preparation was set up in a dark room and the exposed half of the tail was illuminated by a beam of light from an otherwise completely obscured electric lamp. Thus on the same fish one half of the tail was brightly illuminated and the other half was in full darkness. The fish, which remained normally alive and active under these conditions, was held in this position for a little over an hour. Its tail was then cut off, immediately fixed expanded in hot water at 60° C. and prepared for microscopic examination. In all, three such preparations were made. To the unaided eye the two halves of the tail in each of these were indistinguishable in tint and a microscopic examination of their melanophores showed no consistent differences. I therefore agree with Abramowitz (1936) and with Wykes (1938) that there is thus far no evidence in favor of the direct response of the melanophores in *Ameiurus* to light and I regard this possible factor as negligible in dealing with the color changes in this fish.

Since the appearance of papers by von Frisch (1911) and by Scharrer (1928) the pineal organ has been an object of interest to students of the color changes in the lower vertebrates. Wykes (1938) opened the cranium of *Ameiurus* from the dorsal side and destroyed the pineal organ and a part of the diencephalon with a hot needle, but found that after the fish's recovery its color responses to light persisted normally. She pointed out that the roof of the skull and the skin in *Ameiurus* were so dense that the brain seemed well protected from light. She therefore doubted if the pineal organ in *Ameiurus* was functionally concerned with color changes.

Although the roof of the cranium of the catfishes with which I worked was very dense, as had been remarked by Wykes for her own material, this part of the catfish is by no means impervious to light, as can be seen by holding a preparation of the roof of the head between one's eye and an electric light. To prepare catfishes for pineal tests I cut the integumentary nerves to the top of the head to eliminate the possible action of photoreceptors in the skin of that part and then loosened the skin over the head so that a light-proof, thin, metal sheet could be slipped under the skin to cover fully the pineal region. Such fishes were then subjected to bright local illumination over the pineal organ with and without the light-proof sheet in place. In none of these tests did I

notice any change in tint in the experimental fish as compared with a second fish bound next to the one tested and used as a control. I therefore conclude that however the pineal organ may function in the color changes of other fishes, it gives no evidence in *Ameiurus* of being concerned with chromatic responses. My opinion is thus in agreement with that of Wykes.

It will be remembered that some years ago Kropp (1929) pointed out that aqueous extracts of the eye-balls of dark-adapted tadpoles of *Rana clamitans* yielded a mixture that when injected into other tadpoles induced an expansion of their melanophores. To test this possibility in the catfish I ground to a pulp in a mortar with sand and 2 cc. of Ringer's solution ten pairs of eyes from dark catfishes, extracted the juice and injected 0.5 cc. of this juice into a pale catfish and an equal amount into a dark one. I also made a similar extract from the eyes of ten pale catfishes and injected equal volumes of this extract into a pale and into a dark fish. Careful inspection, however, showed no change whatever in the colors of the recipients either local or general and I was forced to conclude that the eye-balls of *Ameiurus* were probably not concerned in any way as centers for the production of hormones of significance in color changes.

This negative evidence from direct stimulation, the activity of the pineal organ, and of the eye-balls as secretory centers is in strong contrast with the positive results from the two other means of activation, the eyes and the integumentary photoreceptors. All workers on the color changes of catfishes have recognized the great importance of the eyes in these responses. Moreover, the necessary presence of another type of receptor, such as the integumentary photoreceptor, has been repeatedly emphasized (Abramowitz, 1936; Matsushita, 1938; Wykes, 1938). To distinguish in an experimental way the parts played by the eyes and the skin receptors in *Ameiurus* is by no means easy. One operational procedure to this end has been found possible. It consists in a complete transverse section of the brain of the catfish in the region of the cerebellum. When such a section is made the portion of the brain anterior to it carries the eyes, the pituitary gland and their connections undisturbed and no other organs of significance for color changes, for the only other components connected with this part of the brain are, in addition to the pineal organ just discussed, the olfactory nerves and the nerves to the eye-muscles. The portion of the central nervous organs posterior to the cut, the hind part of the brain and the whole of the spinal cord, has attached to it all the cutaneous nerves, from the trigeminal to the last spinal. Thus this cut separates completely and perfectly the eyes and their immediate central connections from the nerves

to the whole of the skin and their immediate centers. Such a preparation has its limitations, but is of great importance in determining the rôles played by the two chief systems of chromatic receptors at present under consideration.

The operation for the transverse severance of the brain of the catfish is carried out without great difficulty. The cut is made in a plane transverse to the longitudinal axis of the fish and a little posterior to the anterior edges of the pectoral fins. In executing this cut in a catfish a small aperture is bored through the skin and bony roof of the cranium of the fish on the dorsal median line and in the transverse plane already described. A scalpel with a very narrow, long blade is then inserted through this aperture and with one transverse cut to the right supplemented by a second one to the left the brain may be completely severed cross-wise. In the catfish the brain is not always uniform in location in reference to external landmarks. A transverse cut through the brain, however accurately made, may therefore not always fall in the desired region. If the cross-cut is a millimeter or so too far anterior, the whole optic and pituitary organization may be interfered with and the preparation thus rendered worthless. If it is too far posterior, it may disrupt the medulla oblongata and disturb the respiratory centers to such an extent that the fish will quickly die. If, however, the cut is successful, the desired separation in the brain may be satisfactorily accomplished and the fish after a few days will be ready for tests. I was fortunate in this operation in about one in every four or five fishes. This rather heavy loss was due, I believe, to the considerable variation in the position of the brain in relation to the external topography of the fish as already noted. Fishes successfully operated on commonly lived for from three to four weeks. They were quite active, but their swimming was disorganized and their orientation completely lost. These motor disturbances were doubtless due to the partial destruction of the cerebellum. Such fishes were, however, well adapted as material for chromatic tests.

A pale catfish, when operated upon as already described, will remain pale apparently indefinitely even in a black-walled, illuminated aquarium. One of my fishes lived thus for 24 days after its brain had been transected and died finally because of further operations. As might be supposed, these fishes remain indefinitely pale on illuminated, white backgrounds. Since, however, they stay pale in black, illuminated surroundings and with their eyes, pituitary glands and the connections of these parts fully intact, one is justified in concluding that the eyes of the catfish are not the receptors concerned with the discharge of intermedin from the pituitary gland.

Dark fishes with brains transected through the cerebellum remain dark irrespective of their surroundings and in their unchangeableness resemble pale fishes. The brain transection, in fact, appears to reduce both kinds of fishes to a stage of color immobility. They are in truth, as I have elsewhere shown (Parker, 1940*b*), in a state of chromatic rest. This brain operation appears to render the whole melanophore system of the catfish inoperative. That the melanophores of such fishes are not injured by the transection but are merely rendered quiescent can be shown by the injection of intermedin into a pale operated catfish whereupon it will darken, and the injection of adrenaline into a dark one which will then quickly blanch. These observations lead to the conclusion that the eyes of the catfish are not concerned with the discharge of intermedin.

Further evidence as to the control of intermedin in catfishes can be obtained from individuals whose central nervous organs are intact. When both eyes are removed from a normal, pale catfish and it is allowed to remain in the light, it soon assumes a coal-black tint irrespective of its background (Table I, 9, 10). Such a fish will apparently retain this maximum dark hue for an indefinite period (Van Heusen, 1917; Bray, 1918; Pearson, 1930; Parker, 1934; Abramowitz, 1936; Odiorne, 1937; Wykes, 1938; Osborn, 1938*b*). If a coal-black fish, rendered so by having been blinded, is transferred to darkness, it will cease to be coal-black, but will maintain a very dark tint (Table I, 12).[1] If a pale fish is completely enucleated and put at once into the dark, it will remain pale (Table I, 11). Both these fishes, however, when brought into the light soon become coal-black. I have shown elsewhere (Parker, 1940*b*) that the condition of the melanophores of such fishes in darkness is that of rest and that this stage of rest may be assumed anywhere between one extreme or the other in the whole range of color change. This stage of melanophore inactivity in darkness shows that the photoreceptors in the skin of such fishes are also in quiescence, for all such *Ameiurus* when brought into the light darken to full coal-black. In darkness, then, the photoreceptors in the skin of the catfish must be unstimulated, but when light falls upon them they are activated and thus induce the discharge of intermedin whereby the fish assumes a coal-black tint. That this darkening is due to a blood-borne agent is seen in the fact that when this test is carried out on a fish with a blanched, caudal band, the band darkens at the same time that the fish does.

[1] This statement is not wholly in agreement with the declarations of other workers. I have discussed these differences elsewhere (Parker, 1940*a*) and I shall refer the reader to this discussion for my reasons for the conclusion that blinded, coal-black catfishes in darkness are of very dark tints though not coal-black.

These observations lead to the conclusion that when light falls upon the integumentary photoreceptors of *Ameiurus* nervous impulses are set up for the discharge of intermedin and the consequent darkening of the fish (Abramowitz, 1936). They also show that these photoreceptors are fully quiescent in darkness. I therefore conclude in general that the discharge of intermedin is not the result of impulses from the eyes, but of impulses from the integumentary photoreceptors and that these receptors are stimulated to this effect when light falls upon them but not when they are in darkness.

From this standpoint the one chromatic activity of the photoreceptors in the catfish skin is to induce the discharge of intermedin. That this general control is a reflex operation there is every reason to assume. That it is not a nervous, spinal-cord reflex is clear, for no changes in color can be induced in a spinal catfish by the illumination of the skin on its flanks. In this my observations entirely agree with those of Wykes (1938). However, I do not agree with this worker in assuming that the reflex under consideration is a brain reflex in the way implied by her. Wykes assumes a type of reflex based upon an interpretation of the expanded state of melanophores in denervated pelvic fins whereby the color cells therein located are supposed to be in a condition of dispersed pigment in consequence of their liberation by the denervating cut from some central nervous activity. But this interpretation has already been shown to be untenable (Parker, 1934, 1940*a*). It is true that the reflex here discussed is, as Wykes remarks, a reflex that involves the brain, but the activating impulses are not reflected from that organ over nervous paths to the melanophores as she believes. As already shown, they impinge in the brain on the pituitary gland there to induce the discharge of intermedin. In this type of reflex only the afferent arm is nervous (from the integumentary photoreceptor to the pituitary gland), the efferent arm being humoral (from the pituitary gland by way of the blood and the lymph to the melanophore). I have recently called attention to this somewhat unusual type of reflex, part nervous and part humoral, and I have designated it in consequence of the different conditions of its two arms as a neurohumoral reflex (Parker, 1940*c*; 1940*d*). In conclusion it may be said that the discharge of intermedin by the pituitary gland in *Ameiurus* according to this general view is not excited through the eyes, but through the photoreceptors in the skin and by these photoreceptors only when they are illuminated, not when they are in darkness. Under such circumstances they are inactive.

If the skin photoreceptors in *Ameiurus* are limited in their chromatic function to exciting a discharge of intermedin from the pituitary

gland as a means of darkening the fish, what part do the eyes play in these color changes? It has already been shown that the eyes are not concerned with the discharge of intermedin, for a pale catfish with a transected brain but with eyes and pituitary gland intact will remain pale on a black, illuminated background. Do the eyes control the autonomic nerve-fibers by which the melanophores are made to disperse or to concentrate their pigment?

This question has been in large part answered by the work of previous investigators. The presence of dispersing fibers in the catfish, notwithstanding the doubtful expressions voiced by Osborn (1938*b*) and by Wykes (1938), appears to be well established on the basis of the revival of caudal bands (Parker, 1934; 1940*a*) and of the regeneration of caudal pigmentary nerves (Abramowitz, 1935). Dispersing fibers have been taken for granted in two of the most recent papers on this subject (Chin, 1939; Chang, Hsieh and Lu, 1939). Are these fibers activated from the photoreceptors in the skin, from the eyes or from both sets of receptors? This question cannot be approached from the standpoint of the fish with transected brain, for the cut in such an animal severs not only the nervous tracts from the skin to the pituitary gland, but also those from the eyes to the melanophores. It is best dealt with in hypophysectomized catfishes. Hypophysectomized catfishes lose the intermedin from their blood only slowly. According to Veil (1937) they blanch in about three days. Osborn (1938*b*) identified intermedin in the blood of catfishes by means of tests on pale fishes 70 hours after hypophysectomy. By testing the blood of hypophysectomized fishes on pale individuals or on catfishes of intermediate tint, I have found evidences of intermedin at three days after hypophysectomy but never after five days. When catfishes completely free from intermedin were needed for experimental purposes they were held as a rule for a full week after hypophysectomy before special tests were made upon them. As already stated, a hypophysectomized catfish thus seasoned is limited in its color responses. It is incapable of becoming fully dark (Parker, 1934; Osborn, 1938*a*, 1938*b*; Wykes, 1938; Enami, 1939) or fully pale (Veil, 1938; Enami, 1939; Parker, 1940*a*). In normal *Ameiurus* the fully dispersed macromelanophores, as already noted, have a diameter of about 145 microns and the fully concentrated cells one of about 40 microns. After hypophysectomy the diameter of these cells at maximum dispersion is about 70 microns according to Osborn (1938*b*) or about 100 microns according to my measurements (Parker, 1940*a*) and their minimum diameters some 50 microns. Thus by hypophysectomy the catfish has lost about half its range in color change. The half range that remains to the fish after the loss of its

pituitary gland must be attributed to its other chromatic activators which are in the main the nerves for the dispersion and the concentration of its pigment. The dispersing nerves, supposed by some workers (Parker, 1934; Wykes, 1938) to be the chief agents in darkening the fish, have been shown to be of secondary importance as compared with intermedin (Veil, 1937; Osborn, 1938*a*, 1938*b*; Parker, 1940*a*). The concentrating nerves have long been recognized as the main blanching agents. These general features of the catfish melanophore system must be kept in mind in the following discussion which will be concerned chiefly with the steps necessary for the activation of the two sets of nerves for the color responses.

If a catfish previously hypophysectomized is completely enucleated the system controlling its melanophore responses will be found to be reduced to the photoreceptors of the skin and their nerves, and the two sets of dispersing and concentrating melanophore nerves. These parts together constitute all that is necessary for a complete melanophore reflex. Are these parts so organized that such a reflex is possible?

Catfishes prepared in this way and allowed to recover fully from the operations live well for some weeks. They are very satisfactory material for color tests. Their general conditions are recorded in Table I, 13 to 16. When a hypophysectomized catfish is enucleated even though it is in the beginning as moderately pale as such a fish can be, it is likely to darken considerably as a result of the operation. This is probably due to handling (Parker, 1940*a*). After such treatment these fishes are as a rule about intermediate in tint (Osborn, 1938*b*; Wykes, 1938) though some of them remain paler. In the light they retain their particular tints apparently indefinitely and this retention is irrespective of the background on which they rest (Table I, 13, 14). If hypophysectomized catfishes as near pale and as near intermediate in tint as they can be made to assume by background adjustment are quickly and carefully enucleated and at once put in darkness, they retain their original tints without change so far as can be judged (Table I, 15, 16). Moreover, when they are brought into the light they do not seem to alter in color. All these observations point to hypophysectomized, eyeless catfishes as chromatically inert and open to almost no reflex color change whatever. Their melanophores are in what I have regarded as stages of rest (Parker, 1940*b*). That these color cells have not suffered any serious incapacity by the operations to which the catfish may have been subjected can be shown in their quick and characteristic responses of pigment concentration to injections of adrenaline and of dispersion to pituitary extract (Osborn, 1938*a*). What is lacking apparently in these inert fishes is the reflex connections between the afferent photoreceptor

fibers from the skin and the efferent dispersing and concentrating fibers to the melanophores. In the catfish the nerve connections left after the removal of the pituitary gland and the eyes are not organized for color reflexes and I therefore conclude that the autonomic dispersing and concentrating nerve-fibers of the melanophore system in *Ameiurus* are not open to activation by the photoreceptor fibers from the skin of the fish. These two sets of melanophore fibers, as has long been known, are activated through the eyes and if the results just detailed are correct these receptors are the only ones concerned with the activation of these two kinds of nerve-fibers.

Discussion

The melanophore system in *Ameiurus,* as represented by its macromelanophores, appears to be organized upon the following general plan. The chief receptors concerned with the activation of the melanophores are the eyes and the photoreceptors of the skin. The photoreceptors are stimulated not in darkness but only when light falls upon the skin and are not influenced by an experimental environment, black or white. They excite the discharge of intermedin from the pituitary gland and the consequent darkening of the fish. They are not concerned with the activation of the chromatic nerve-fibers, dispersing or concentrating. The eyes are receptors through which a background, white or black, makes itself felt in the system of color cells. Through the eyes are excited the concentrating autonomic nerve-fibers and the dispersing ones by which the pigment in the melanophores is gathered in the center of the color cell or scattered throughout its processes, blanching or darkening the fish. The eyes, therefore, are the receptors concerned with the discharge of adrenaline and of acetylcholine from the chromatic nerve terminals. The eyes are not so related to the discharge of intermedin, and yet it would probably be false to deny to them any influence over the pituitary gland. When a catfish becomes pale through the entrance into its eyes of light reflected from a white surface, its skin is also under reasonably bright illumination. The effect of this skin illumination would be to induce the discharge of intermedin whereby the fish would be darkened. There is, however, not the least evidence to show that in a brightly illuminated, white environment any darkening agent is present in a catfish. On the contrary, the fish under these circumstances is fully and completely pale as though under the undivided control of adrenaline. It is conceivable, of course, that there is under these conditions a neurohumoral competition between intermedin and adrenaline like that which has already been pointed out in the dogfish *Mustelus* (Parker, 1937),

but evidence to this effect, unlike that seen in the dogfish, is entirely lacking. It is much more probable that in illuminated white surroundings the stimulation of the eye excites only the concentrating nerve fibers and at the same time overcomes the discharge of intermedin due to the illumination of the skin photoreceptors by some action on the pituitary gland. From this standpoint the eye in a luminous white environment not only excites the discharge of adrenaline but probably inhibits that of intermedin. In this respect the eye very likely plays a negative but important part in the control of intermedin. This idea of chromatic inhibition has already been suggested by Zoond and Eyre (1934) and by Sand (1935).

This, in brief, is a rough outline of the melanophore system in *Ameiurus*. That it is correct in its chief features seems probable from the evidence presented. That it is in any sense complete is far from likely. Why should hypophysectomy limit color responses and how is this limitation accomplished at both the dark and the pale ends of the color range? Is excitement pallor (Abramowitz, 1936) a reflex and if so what are the receptors and the nerve paths? The same questions may be asked concerning darkening due to handling. What is the nature of the so-called inherent reactions of melanophores, a subject recently emphasized by several active workers (Abramowitz, 1936; Osborn, 1938*b*)? These and a score of other questions show how restricted our knowledge is not only of the melanophore system in *Ameiurus* but of the chromatic system in general. They invite further research.

Summary

1. The melanophore system in the catfish *Ameiurus* composed of epidermal micromelanophores and of subdermal macromelanophores is activated not through direct stimulation by light, by humors from the eye ball, nor by the activity of the pineal organ, but by nerve impulses received from the eyes and from the photoreceptors in the skin.

2. The skin photoreceptors are stimulated by light but not by darkness and are exclusively concerned with the discharge of intermedin from the pituitary gland whereby the catfish darkens (neuro-humoral reflex).

3. The eyes are differently stimulated by light from a black environment and by light from a white one. In the former they give rise to impulses that pass over the dispersing autonomic nerve-fibers, induce a discharge of acetylcholine, the dispersion of pigment, and the darkening of the fish. In the latter they originate impulses that pass over the concentrating autonomic fibers, induce a discharge of adrenaline, the con-

centration of pigment, and the blanching of the fish. Impulses induced through the eye by an illuminated, white environment probably also inhibit the discharge of intermedin from the pituitary gland.

4. A technique is described for the transection of the catfish brain at the level of the cerebellum whereby the eyes and their appended centers may be separated from the whole integumentary photoreceptor system and its centers.

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THE EFFECT OF GROWTH AND NUTRITION ON MITOCHONDRIA IN LIVER CELLS OF FUNDULUS HETEROCLITUS

MARY BURTON DERRICKSON McCURDY

(From the Marine Biological Laboratory, Woods Hole, Mass. and Milligan College, Tennessee)

Introduction

In a recent study on *Triturus pyrrhogaster* (McCurdy, 1939) I found that starvation and re-feeding tend to produce definite changes in the mitochondrial picture of the liver cells. In the course of starvation, the elongated mitochondria which predominate in well-fed animals gradually give way to granular mitochondria, and these latter finally become predominant. When the procedure is reversed and starved animals are re-fed, the mitochondria change in the opposite direction, from granular to elongated. These mitochondrial changes, however, though they clearly depend upon digestive activity and degree of starvation in general, are more closely related to the amounts of fat and glycogen in the liver itself. It can be stated as a rule that in the liver cells of *Triturus* increase in the fat and glycogen content is accompanied by an increase in the proportion of elongated mitochondria, and decrease in the fat and glycogen content is accompanied by an increase in the proportion of granular mitochondria. There is thus an important relationship between the nutrition of cells and the form of their mitochondria.

The present study is concerned with two questions: (1) Does a similar relationship between mitochondrial form and the amount of storage products exist in a different species? (2) Does growth affect the morphology of the mitochondria?

Material and Methods

Fundulus heteroclitus was selected for this study because it was readily obtainable at Woods Hole during the spawning season and adults and larvae could easily be kept alive in the laboratory.

For the starvation experiment, adults were kept in two aquaria supplied with constantly running sea water. One set were fed regularly every day with clams cut into small pieces, four individuals for one

week and four others for two weeks, and then killed twenty-four hours after their last meal. The second set were denied food, four individuals for two weeks and four others for three, before killing. All animals were killed by decapitation, the body opened immediately and small pieces of liver excised and fixed.

For the growth experiment, larvae were obtained by stripping adult males and females and fertilizing the eggs. At frequent intervals the hatching larvae were collected and removed to separate bowls labelled with the time of collection. After the first twenty-four hours they were fed every day with bits of clam, finely macerated. Each day, from the time of hatching through the twenty-fourth day, a batch of larvae were fixed whole.

The fixatives employed were Regaud for mitochondria and fat and Lison's mixture (dioxan-picric acid-acetic acid-formalin) for glycogen. The stains were: for mitochondria, Heidenhain's iron-haematoxylin; for fat, using frozen sections, Sudan III; and for glycogen, Best's carmine. The methods were the same as those used and described in detail in the paper referred to previously.

Observations

A. Starved and Fed Adults

In all the well-fed adults elongated mitochondria are predominant, chiefly long filaments, some branched or racquet-shaped, though a few large granules are present. Fat and glycogen are abundant.

In all the starved adults granular mitochondria are predominant, though there are a good many beaded forms, and a few rods. Fat and glycogen are totally absent.

B. Growing Larvae

Mitochondria.—In larvae just hatched (average length 4 mm.) granular mitochondria predominate, though a few thick rods and drop-like forms are present. With larvae of increasing age there is a gradual increase in the number of elongated mitochondria until at twelve days (average larval length 6.5 mm.) these forms predominate. By twenty to twenty-four days (average larval length 7 mm.) the elongated mitochondria are filamentous and resemble the mitochondria of well-fed adults. Beaded mitochondria seem to be transitional forms, as in *Triturus,* since they do not exist in the newly-hatched larvae but begin to appear later and then disappear as the smooth elongated forms come into prominence.

Fat.—There is no fat in the liver cells until the third day after hatching, when a small amount appears in the cells adjacent to the blood capillaries. Previous to this stage the fat is limited to the yolk and the blood stream. Fat increases in the liver cells gradually from the third day on and by the twelfth day is abundant. The yolk persists until about the seventh day, decreasing gradually.

Glycogen.—No glycogen is present in the liver at hatching. It appears in small amounts after a day. Like the fat it gradually increases in amount and is quite abundant by the twelfth day.

CONCLUSIONS

It appears from the foregoing observations that in the adult *Fundulus* mitochondrial form is correlated with the presence or absence of storage products in exactly the same way as in adult *Triturus pyrrhogaster*.

Larvae just hatched have granular mitochondria in the liver cells as do starved adults. As they grow, elongated mitochondria appear and finally become predominant, as in the well-fed adult. Since, moreover, newly-hatched larvae have no fat or glycogen in the liver, but as they grow older fat and glycogen appear and increase in amount for some time, it can be stated that the course of change in regard to fat, glycogen and mitochondrial form during normal growth in *Fundulus* is the same as in the case of starved adults restored to normal by re-feeding.

The rule which applies to mitochondrial form in *Triturus pyrrhogaster* holds good for both adults and larvae of *Fundulus heteroclitus*. The form of the mitochondria stands in a definite relationship to the amount of storage products, and thus serves as an index of the nutritional state of the animal.

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GROWTH STUDIES ON CILIATES

VI. Diagnosis, Sterilization and Growth Characteristics of Perispira Ovum

VIRGINIA C. DEWEY [1] AND G. W. KIDDER

(Arnold Biological Laboratory, Brown University and the Marine Biological Laboratory, Woods Hole, Mass.)

The growth characteristics of a number of species of ciliates have been reported recently in the literature. Most of these papers deal with saprozoic forms (Phelps, 1935, 1936, etc.) or with bacteria-feeders (Johnson, 1933; Kidder and Stuart, 1939*b*). The report which is to follow deals with observations of a quantitative nature made on the holotrichous ciliate, *Perispira ovum,* and represents the first attempt at an anlaysis of the growth characteristics of an obligate carnivore in bacteria-free medium, although Brown (1940) has published a brief account of some of the growth characteristics of *Leucophrys patula,* a facultative carnivore. Due to the fact that *Perispira* is not a common genus and its previous descriptions have been contradictory (Stein, 1859; Levander, 1894; Kahl, 1926), a rather complete description of its organization will be given. This description will serve to establish the identity of our experimental organism and should eliminate confusion in future investigations, regarding its specific designation. We agree with the views expressed by Taylor and Furgason (1938) and Furgason (1940) that the space devoted to a purely morphological description is warranted as a means of standardization of experimental material.

Material and Methods

During the summer of 1939 a number of specimens of *Perispira ovum* were found in a sample taken from a fresh water stream on Gifford St. in the town of Falmouth, Massachusetts. This sample contained many *Euglena* along with numerous other species of protozoa. The *Perispira* were isolated and maintained in mass culture, along with their associated bacteria and *Euglena gracilis,* for a period of two months before any attempt was made at sterilization. During this period care had to be taken to make frequent sub-cultures, as heavy bacterial growth proved to be detrimental to the *Perispira.*

[1] Aided by a grant from the Manufacturers' Research Fund for Bacteriology and Protozoölogy at Brown University.

Description of Perispira ovum

The ciliate is a member of the family Spathidiidae. It is evenly oval in shape, its size varying with the state of its nutrition. Well-fed organisms measure 65 μ–120 μ in length $\times$ 50 μ–110 μ in width, while starved ciliates are much smaller (30 μ–60 μ $\times$ 20 μ–45 μ). The most characteristic structure of the body is a spiral ridge, originating at the dorsal anterior portion of the body. From the point of origin the ridge bends sharply to the right and then spirals posteriorly toward the left, ending on the left side of the body near the posterior end (Fig. 1).

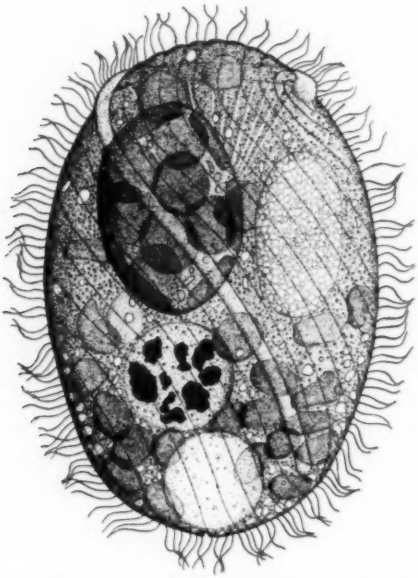

FIG. 1. Living ciliate, left side $\times$ 1000. The slender refractile trichites may be seen extending from the anterior end into the endoplasm. To the right of the drawing is the macronucleus; toward the posterior end are the contractile and excretory vacuoles, the excretory pore and the scattered chloroplasts and paramylum bodies. A single *Euglena* has recently been ingested.

Thus the ridge describes only one complete turn about the body, whereas Kahl (1926) figures an extra half turn. This ridge is composed of structureless, homogeneous protoplasm throughout most of its length, but in the anterior third it bears a narrow groove. This groove is slightly expanded very near its beginning into what appears to be a

small pore (Fig. 2). The pore and the groove represent the mouth, which is open only during the few seconds of food ingestion. The mouth can be seen in the non-feeding organism only after appropriate preparation (opal blue or nigrosin). Extending from the mouth and running into the endoplasm are a number of refractile and delicate trichites (Fig. 1). In most living specimens a few of these trichites may be seen lying loose in the endoplasm, but the majority are arranged as are those in a typical *Spathidium*. We have been unable to demonstrate them with any of the standard techniques. Levander (1894)

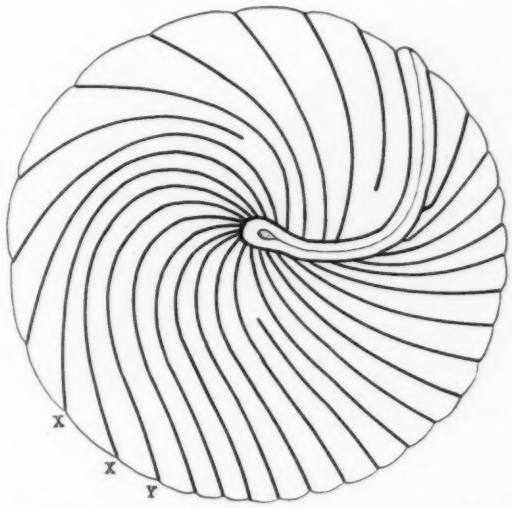

FIG. 2. Diagrammatic representation of the anterior pole, showing the ridge and the origins of the ciliary lines. The mouth is indicated in the ridge. *x*, *x* represent the ciliary lines which bear the paired, short bristles. *Y* represents the ciliary line which bears the long, unpaired bristles.

saw these trichites and included them in his figure but interpreted them as forming a basket. Kahl (1926) figures the trichites but refers to them as "Trichocysten" and describes them as being present in the protoplasm of the ridge. We have been unable to verify this last observation and there is no evidence from a study of the activity of a feeding animal that it possesses any trichocysts which function as do those in *Spathidium*.

The disposition of the cilia may be observed clearly in small organisms which are devoid of food. Relief staining methods with opal blue or nigrosin on medium-sized or small ciliates give excellent and

striking results and permit accurate analysis of the relative size and distribution of the motor organelles (Fig. 3, *A* and *B*).

On either side of the spiral ridge there is a row of closely set cilia. These rows join at the anterior end of the ridge but remain separate at its posterior end. This arrangement makes, in effect, a continuous row of cilia starting at the posterior end of the ridge, encircling the anterior end, and returning on the opposite side of the ridge. Contrary to the statement of Kahl (1926), we have found these cilia to be identical in size but much more closely set than the rest of the peripheral

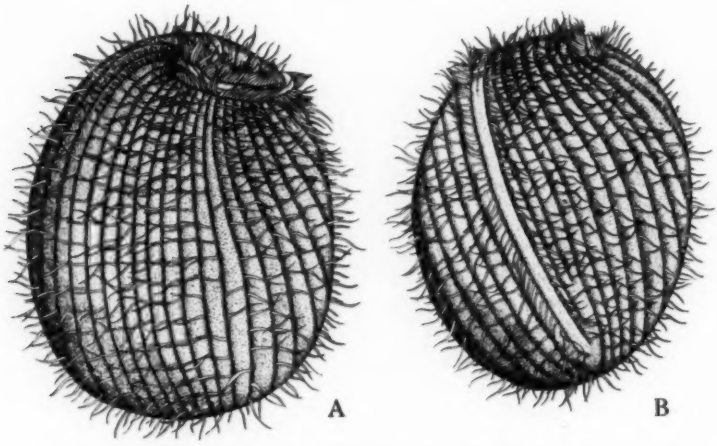

FIG. 3. Drawings of opal blue preparations of medium-sized organisms to show the size and arrangement of the cilia and the disposition of the ciliary rows. Organisms slightly flattened in preparation. × 700. *A*. Right side. Note the three rows, bearing bristles, toward the left of the drawing. *B*. Left side, showing the ridge.

cilia. It seems probable that these closely set cilia were mistaken by Kahl for the trichocysts which he describes as being present in the ridge (Fig. 3, *A*).

The general peripheral ciliary rows originate from the edges of the ridge in the region of the anterior pole. These rows spiral around the body from right to left, paralleling the ridge. They end irregularly in the region of the posterior pole. There are 26 complete rows of fairly widely placed cilia, with from one to three interpolated rows. The general arrangement of these rows may best be understood by referring to Fig. 2. Three of these peripheral rows deserve special attention, since they are made up of diverse types of structures. Two of the rows,

originating from the anterior tip of the ridge, bear short, paired bristles (Fig. 2, *x, x;* Fig. 3, *A* and *B*). These bristles are distributed along the first quarter of the length of the row and appear to originate from basal bodies directly in the row. They may be observed in living organisms and are clearly demonstrated after relief staining. They appear to vibrate rapidly but through extremely short arcs. They correspond to the " Chemoreceptoren " described by Gelei (1933) in *Trachelophyllum,* except that he maintains that their origin is between the ciliary rows. We cannot subscribe, at present, to Gelei's theory as to their function. There is no evidence that *Perispira* receives chemical stimulation through these bristles. Just to the right of the rows which bear the short bristles is a single row of long, stiff bristles (Fig. 2, *y;* Fig. 3, *A* and *B*). These are unpaired and occupy most, but not all, of the length of the row, which is completed posteriorly by normal flexible cilia. These long bristles correspond to the " Tastborsten " of Gelei (1933), but do not appear to function as tactile receptors, as he supposed they did in *Trachelophyllum.* Contact with prey in the region of these long bristles evokes no apparent response in *Perispira.* Another region where touch stimuli are received will be described shortly when the mechanism of feeding is considered.

The endoplasm of *Perispira* is coarsely granular and contains many different types of inclusions. Well-fed organisms are bright green due to the tightly packed chloroplasts of the ingested *Euglena.* The protoplasm of the *Euglena* is digested first, releasing the chloroplasts and the paramylum bodies. Next the chloroplasts are broken down and the unassimilated material deposited in a vacuole, as a reddish-brown mass, for defecation. Animals in the early stages of starvation contain quantities of paramylum bodies, which gradually disappear, leaving the *Perispira* relatively clear. These paramylum bodies appear to function as reserve food, enabling the ciliate to continue living long after all the *Euglena* have been ingested.

A single contractile vacuole is located at the posterior end of the body. It empties its contents to the outside through a permanent pore, which is always visible at the extreme posterior pole (Fig. 1).

The macronucleus is quite large and coarsely granular. It is variable in shape, being ovoid to elongate or even horseshoe-shaped. In starved animals it regularly breaks up into two spheres, as described by Levander (1894). After staining with haematoxylin, two types of granules are demonstrated, the fine chromatin granules and larger spheres of deeply-staining substances lying in vacuoles. Their appearance corresponds to that of the spheres found in such forms as *Paramecium*

(Wenrich, 1926) and *Diophrys* (Summers, 1935). After the Feulgen reaction, however, only the chromatin granules are stained and the vacuoles which hold the larger spheres appear hollow, giving the macronucleus an areolar appearance.

There is a single micronucleus in *Perispira ovum* which is very low

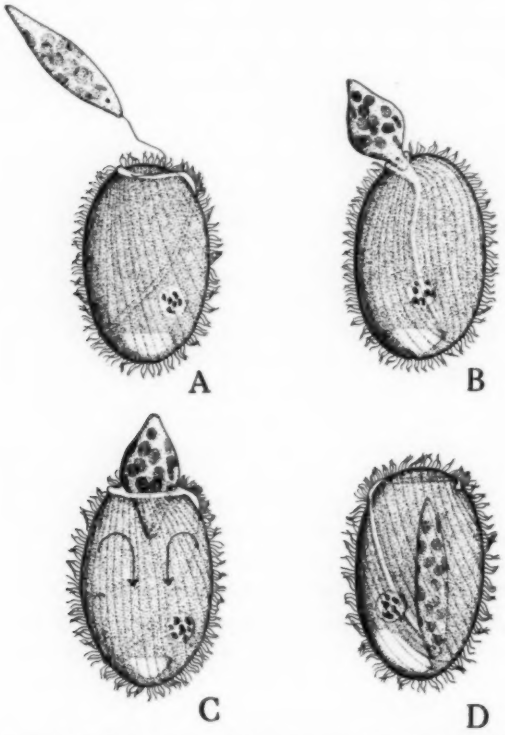

FIG. 4. Starved *Perispira ovum*, showing successive stages during the ingestion of a *Euglena*. *A*. Flagellum is caught. *B*. Mouth beginning to open. *C*. Arrows indicate the direction of the cyclosis which carries the *Euglena* in. *D*. Mouth closed immediately after ingestion.

in chromaticity. It regularly lies very close to the macronucleus and may easily be overlooked. Kahl (1926) states that he was never able to identify it with certainty.

We have made extensive observations on the mechanism of feeding. Food taking is best observed by placing a drop of a culture containing starved *Perispira* on a slide and adding to this drop a number of active

Euglena. Within a few seconds almost all of the ciliates will have started feeding and the whole process can be studied under an oil immersion lens.

The ciliates swim with a rotating motion which is uninterrupted by contact with other ciliates or with rounded *Euglena.* When the flagellum of an active *Euglena* comes in contact with any part of the ridge, however, the motion of the *Perispira* changes and it stops its rotation. If the contact has been made near the anterior end of the ridge (Fig. 4, *A*), the flagellum is rapidly drawn in, dragging the flagellate up to the mouth. The ridge immediately opens along the seam (Fig. 4, *B*) and the *Euglena* is drawn into the body of the *Perispira* (Fig. 4, *C* and *D*). The mouth then closes and the whole process may be repeated many times in the course of a few minutes. The force which draws the prey into the mouth is manifested by the increased intensity of the cyclosis of the protoplasm of the ciliate, which creates a current directed inward from the mouth opening (Fig. 4, *C*). Ingested *Euglena* are not surrounded by a distinct vacuole, but appear to lie free in the endoplasm of the ciliate. Killing of the prey is very slow and it is not uncommon to see four or five ingested flagellates contracting and expanding within the body of the *Perispira.*

Although we placed *Perispira* in fluid containing numerous species of protozoa, we have never observed it ingesting any types except euglenoid flagellates. It may be that its specialization has gone so far that its prey must possess certain structural characteristics in order to evoke the feeding response.

Sterilization and Establishment in Bacteria-free Culture

Advantage was taken of the fact that well-fed *Perispira* are positively geotropic. One to two milliliters of a heavy suspension was placed in the top of a special tube filled with sterile water (pH 7.0). The tube was constructed with alternating shelves in order to prevent any clumps of débris from falling to the bottom. After the majority of the ciliates had migrated around the shelves to the bottom of the tube they were drawn off into a sterile, cotton-plugged centrifuge tube. The ciliates were then centrifuged slowly until concentrated, the supernatant fluid withdrawn with a sterile serological pipette and more sterile fluid added. This washing process was repeated ten times, after which the ciliates were placed in the top of a 50 ml. burette full of sterile water. After this second migration had been completed and a large number of the ciliates withdrawn from the bottom of the burette into a sterile cotton-plugged tube, they were placed in a Syracuse watch glass

enclosed in cellophane (Kidder, Lilly and Claff, 1940). Single ciliates were then withdrawn by means of sterile capillary pipettes and placed into other similar dishes, from which they were then removed to tubes containing either water or 0.5 per cent Yeast Harris.

The above method yielded several sterile ciliates. The various steps described were necessary, inasmuch as this ciliate possesses rather uneven contours and is not easily washed free of its adhering bacteria. All cultures finally labeled as sterile were so designated only after the various sterility tests had been used in accordance with our previously described methods (Kidder and Stuart, 1939*a*).

The washed ciliates were placed into: (1) water (pH 7.0) or (2) 0.5 per cent filtered Yeast Harris. To both of these fluids sterile *Euglena* had previously been added. The method of sterilization of our strain of *Euglena* has been reported elsewhere (Kidder, 1940). Growth of the ciliates was rapid in some tubes but extremely slow in others. It soon became evident that the tubes in which rapid multiplication occurred were bacterially contaminated, while the others were sterile. We considered the possibility that the bacteria might be furnishing some substance needed for normal growth. Accordingly a suspension of *Phytomonas* (the contaminating bacterium from one of the growing cultures) from an agar slant was made in water (pH 7.0) and autoclaved for 20 minutes at 120° C.; 0.5 ml. of this suspension was added to a tube containing sterile ciliates in water with *Euglena*. A Seitz filtrate of a 24 hr. culture of *Phytomonas* in 0.5 per cent Yeast Harris was also prepared and 0.5 ml. added to another tube containing sterile ciliates in water with *Euglena*. In both tubes the ciliates immediately began to multiply and proved to be sterile.

Loop transplants were made from the first of these cultures into: (1) autoclaved *Aerobacter* in casein peptone (Peptone Roche) and (2) autoclaved *Phytomonas* in Yeast Harris to both of which *Euglena* had been added. Growth was good in both media, but in all cases appeared slightly better without Yeast Harris. Transplants from these cultures into Difco Tryptone and *Euglena*, without autoclaved bacteria, proved successful and the cultures have been carried in 1.0 per cent Tryptone since then.

After nearly a year of continued culture in Tryptone and *Euglena*, we feel sure that the function of the autoclaved bacteria and of the bacterial filtrate in our initial establishment experiments was simply to reduce the speed of the growth of the *Euglena* so that the single *Perispira* which were inoculated were not "overgrown." Later serial stock transplants were always made with many *Perispira* and the rapidly growing *Euglena* were quickly reduced in number.

Technique of Experimental Culture

All experimental cultures were grown in the special culture flasks described elsewhere (Kidder, 1941). These flasks are provided with a port plugged with a vaccine tip, which allows one to draw off samples for counting with little danger of contaminating the cultures. The volume of all cultures was 100 ml. Incubation was at 28° ± 1° C. The cultures were grown in a box provided with a large water filter behind which a constant source of light was provided by means of a 50-watt electric bulb.

The fluid to be used in any set of experiments was autoclaved, allowed to cool and inoculated with *Euglena gracilis* and placed in the light box for from 24 to 48 hours. Just before the *Perispira* were inoculated the concentration of *Euglena* was determined by drawing out a sample and making cell counts with a Sedgwick-Rafter counting chamber and a Whipple micrometer. From a culture of *Perispira* two to three weeks old, the average size of the individuals having been determined previously, inoculations were made through the side arm of the flasks. The initial inoculum was determined for each flask by immediately withdrawing a sample and counting the number of *Perispira* in a unit volume. These counts were made with the aid of a dissecting binocular microscope, using the same system as has been reported for *Tetrahymena* (Kidder, 1941). Thereafter samples were taken at regular intervals and the concentrations of *Euglena* and *Perispira* determined. The average individual volume of the *Perispira* was calculated from measurements of 50 cells. Before measuring, the cells were fixed in the fumes of osmic acid. The volume was calculated by considering the individual cell as a prolate spheroid and applying the formula $V = 4/3\ \pi\ ab^2$, where $2a$ = the major axis (length) and $2b$ = the minor axis (width).

Growth

Normal Population Curve

The growth of a population of *Perispira*, when they are inoculated into a flask of 1 per cent Difco Tryptone containing a concentration of *Euglena gracilis* of approximately 10,000 per ml., follows a reproduceable and characteristic course. The shape of the curve depends on a number of conditions, one of which is the age of the inoculum. If *Perispira* reproducing at their maximum rate (logarithmic growth phase) are used as the inoculum, they continue to reproduce at this rate in the experimental flask. When the logarithms of the population densities are plotted against time the result is a straight line. During this

TABLE I

Average data from five experiments. Medium, 1 per cent Difco Tryptone; age of inoculum of *Perispira* 15 days; *Euglena* grown in the medium 34 hours before the *Perispira* were inoculated.

	Time in Hours										
	0	18	30	42	60	90	114	139	162	186	204
Perispira per ml.	100	93	145	235	554	4250	22920	57800	78100	79160	77700
Euglena per ml.	14640	70820	159600	282800	393400	289800	48240	2096	—	—	—
Indiv. Vol. in $\mu 3$	21490	110750	147000	174400	206400	237400	126400	54750	36750	32440	31250

TABLE II

Medium—1 per cent Difco Tryptone; age of inoculum of *Perispira*—21 days; *Euglena* grown in the medium 48 hours before the *Perispira* were inoculated.

	Time in Hours												
	0	23.5	49.5	73	92.5	115.5	145	168	192.5	216.5	403	571	1003
Perispira per ml.	90	75	490	2000	10000	36500	85500	93500	112000	85000	62000	11750	350
Euglena per ml.	9500	53000	130000	255000	241000	11500	—	—	—	—	—	—	—

logarithmic growth phase the generation time (calculated from the formula $g = \frac{t \log 2}{\log b - \log a}$ where t = the time in hours during which the population has been increasing, a = the number of cells per unit volume at the beginning and b = the number of cells at the end of the time, t) is $10.5 \pm .3$ hours. When ciliates taken from the stationary phase (10 to 20 days old) are inoculated there follows a period of approximately

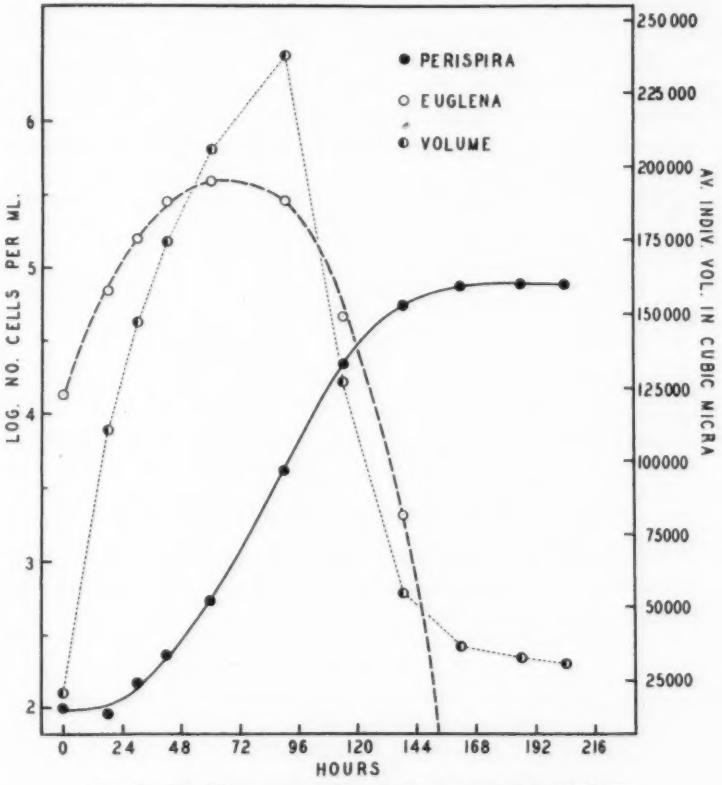

FIG. 5. Graphic representation of data presented in Table I.

24 hours during which there is no increase in the population. Usually some of the inoculated ciliates die during this lag phase. After a considerable number of *Euglena* have been eaten, reproduction follows and the culture goes into the logarithmic phase. The slope of the curve (and therefore the generation time) is identical with that for populations started with logarithmic ciliates (Table I; Fig. 5).

During the early phases of a culture the *Euglena* show a marked increase in concentration but the *Euglena* curve turns over rapidly as the *Perispira* increase. The *Euglena* curve represents the resultant of their own multiplication and their destruction by the *Perispira*. At about the 120th hour most of the trophic flagellates have been ingested (Fig. 5). These curves correspond, in general, to Fig. 6 in Brown's (1940) paper.

After most of the available flagellates have been eaten the *Perispira* continue to reproduce for some time at the expense of their individual size. Eventually the concentration reaches a relatively constant level at about 100,000 cells per ml. From then on the population declines slowly and there are still many small, trophic forms, capable of starting healthy cultures, even after two months. Table II gives the results of

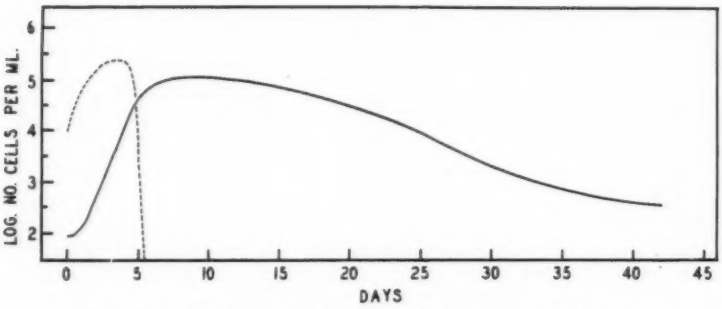

FIG. 6. Graphic representation of data presented in Table II.

an experiment carried for 42 days and these results are shown graphically in Fig. 6. Separate experiments, wherein large, medium-sized and small ciliates were selected for single cell inoculations, indicate that there is a relationship between the size of these starved cells and their ability to resume feeding and reproduction. This correlates with the deaths which occur upon mass inoculations into the experimental flasks. We believe that the variability in size during the late phases of a culture is due to purely fortuitous circumstances, however, and that the larger forms are those which were able to procure the last of the *Euglena*. Further investigations upon this point are needed.

Size Changes in Relation to Age of Culture

Ciliates taken from cultures three weeks old are very small and transparent. As was stated above, when these organisms are used as the inoculum a lag occurs before reproduction starts. It is during the

lag phase that the size of the individuals is increasing most rapidly. Feeding starts as soon as the *Perispira* and *Euglena* are brought together. The ciliates do not have to reach their maximum size, however, before they undergo reproduction. The individual size steadily increases during the logarithmic phase until the time when the food organisms begin to decrease in concentration. After this peak the size diminishes rapidly. The most rapid decrease in cell size corresponds to the upper limits of the logarithmic phase and the phase of negative growth acceleration. During the stationary phase the cell size is still decreasing, but very slowly (Fig. 5). This last and gradual decrease seems to be the result of the utilization of stored food materials while the previous rapid decrease was brought about largely by partition of protoplasm through rapid division.

The size changes hold for all types of media investigated so far and for the two species of euglenoid flagellates employed (*Euglena gracilis* and *Astasia klebsii*) as food organisms. Differences in speed of increase and maximum size attained were noted but these differences were slight.

Effect of Media, Light and Species of Food Organism

Having established the fact that the growth characteristics of *Perispira* in Difco Tryptone and *Euglena gracilis* were quite regular and reproduceable, experiments were set up to determine what effects would be produced by varying the standard combination. Flasks were prepared as before except that 1 per cent Difco Proteose-peptone or 0.5 per cent Yeast Harris was substituted for the Tryptone. In other flasks sterile *Astasia klebsii* was substituted for *Euglena;* still other flasks were prepared with colorless *Euglena* (grown in the absence of light) in Tryptone. All the flasks were inoculated with *Perispira,* the first three types were incubated in the light box while the last was incubated in a constant temperature box (27° C.) in the dark.

The differences observed between the various types of experiments may be summarized briefly. In the colorless *Euglena* there were a few more deaths among the individuals in the initial inoculum than in the controls. The generation times in the logarithmic phase were identical, however, and the maximum concentration and longevity of the cultures were not significantly different. In Proteose-peptone the general shape of the curve was similar to that of the control, but the generation time was 12.2 hours as compared to 10.6 hours for the control. The Proteose-peptone cultures declined more rapidly than did the controls. When the *Perispira* fed on *Astasia* the generation time was greater than

in the controls (12.5 hours), the maximum concentration was much lower (13,000 as compared with 93,000 per ml.) and the decline was very rapid. Moreover the *Astasia* were never completely removed from the culture, as shown by the fact that after the *Perispira* had become reduced to 40 per ml. (17 days) the *Astasia* had multiplied so that the concentration was again appreciable. These declining ciliates refused again to feed on the *Astasia*. In Yeast Harris the ciliates inoculated had nearly all died in 24 hours and in 48 hours all were dead.

General Observations

There are a number of comments to be made which do not have a place in the experiments described above.

When *Perispira* was first isolated and was growing in association with bacteria many monsters invariably appeared in the heavy cultures. These monsters were sometimes two to three times the size of the largest normally shaped individuals and were quite irregular in outline. They appeared to be the result of a failure to divide, on the part of certain individuals, while the power to ingest food was retained. After the establishment of our cultures in bacteria-free media only occasionally were monsters observed. When these organisms were encountered they were not used either in the counts or in the measurements. Attempts to start cultures from these monsters always failed.

Although there is no evidence that *Perispira* forms cysts, conjugates or undergoes endomixis, a peculiar type of reaction is characteristic of organisms in the later stages of a culture. About the seventh day after a culture is started a small percentage of the individuals become associated in pairs, superficially resembling conjugants. Upon closer examination, however, no protoplasmic fusion or even contact can be detected. Instead the long cilia of the anterior end of one individual appear to be stuck to the anterior portions of the ridge of the other, thereby holding the two organisms together. This association is rather loose and the two separate soon after they are placed on a slide. We do not know whether or not this reaction has any significance in the life history of the ciliate, but we are inclined to think that it may represent simply an increased stickiness of the ridge-ectoplasm during the initial period of food stortage.

We have evidence that indicates that the establishment of the normal reproductive rate of a freshly inoculated culture of *Perispira* is influenced by the time during which the food organisms had been growing in the medium before the inoculation of the ciliates. This is a conditioning effect and is being investigated for a future report. It is also

true that the physiological condition of the food organism determines, to a large extent, the ability of the *Perispira* to feed. Recently we have had cultures in which the *Euglena* (grown through many transplants in 1 per cent Difco Tryptone) were smaller than normal and possessed a reduced number of chloroplasts. Although these *Euglena* were active, the *Perispira* ingested few of them and as a consequence remained relatively small and reproduced very slowly. When these ciliates were presented with normal flagellates, however, they began to eat rapidly. In order to keep our food organisms in good condition, therefore, it seems necessary to maintain stocks in an acetate-containing medium, such as Medium D described by Hall (1937).

Discussion

The establishment of *Perispira ovum* in bacteria-free culture along with *Euglena gracilis* as the food organism affords an opportunity for the study of a carnivore under controllable conditions. The fact that cultures remain alive for only a short time when in association with their natural bacterial flora, while sterile cultures last for months, emphasizes again the harmful effects of some types of bacteria. This situation was pointed out in a previous work (Kidder and Stuart, 1939*a*) on a bacteria-feeding species and seems to hold equally well for this carnivore.

An analysis of the reproductive rate and the rate of increase in cell size is interesting. The lag phase is not a period of inactivity. The ciliates are feeding rapidly and increasing in size at a higher rate than at any other time. This is also true of the majority of bacteria (Henrici, 1928), although the mechanism of size increase appears to be different (for a discussion of this point see Stephenson, 1939). A similar analysis of the size changes of *Leucophrys* would be interesting. No data on this point are given in Brown's (1940) paper. During the first part of the logarithmic phase the size measurements indicate that reproduction, while it certainly decreases cell size (by one half), does not keep pace with individual growth resulting from feeding. These ciliates may be said to eat faster than they divide. After the concentration of the food organism begins to decline there is no decrease in reproductive rate, so that, for the first time, reproduction overtakes and passes feeding rate. Experiments wherein food organisms would be continually added to the culture would be very interesting in this connection. Such experiments are planned.

Factors limiting the growth of carnivorous ciliates, grown as described above, have not been analyzed. Under the conditions of our

experiments perhaps the most important single factor is food, but there are many others. As compared to a culture of a purely saprozoic species these carnivores offer certain advantages and certain disadvantages for growth studies. One of the most complicated factors is an evaluation of the balance between the growing food organism and the carnivore. We will be nearer a solution of some of the problems when we formulate a medium which will not support multiplication of the food flagellates, but will maintain them in a trophic condition. So far none of the media used satisfies these requirements.

Summary

1. *Perispira ovum* is a member of the family Spathidiidae. It is carnivorous, feeding on euglenoid flagellates.

2. A morphological description is given in order to establish the identity of the experimental organism.

3. A description of the mechanism of feeding is given. *Perispira* possesses a spiral ridge to which the flagella of euglenoid flagellates adhere. The flagellum and then the body of the prey is drawn into the mouth of the ciliate where it eventually rounds up and is digested. The cytoplasm of the prey is digested first, then the chloroplasts and lastly the paramylum bodies.

4. *Perispira* was rendered bacteria-free and established in culture with sterile *Euglena gracilis* as food.

5. When the population of such a culture is followed, a typical growth curve results. If ciliates from an old culture are used as the inoculum there follows a period of lag, a logarithmic growth phase, a phase of negative growth acceleration, a stationary phase and a phase of slow decline.

6. Cell size increases during the lag phase and early logarithmic phase but decreases rapidly during the late logarithmic phase and phase of negative growth acceleration. Cell size is correlated with the presence of food and the rate of cell division.

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THE DEVELOPMENT OF A COLONIAL ORGANISM: SYMPLEGMA VIRIDE

N. J. BERRILL

(Department of Zoölogy, McGill University, Montreal)

The compact, closely related group of polystyelid ascidians, including the botryllids, are all able to produce buds, either to form social aggregates or true colonies. In either case the manner of budding is fundamentally the same, namely, by the protrusion and development of localized regions of the atrial epithelium together with its overlying epidermis. At the same time, the nature and form of the colonies or groups are intimately related to the differences, with regard to this phenomenon, that do exist among the various genera. A comparative study of bud production and bud development within the polystyelids is illuminating, and of these ascidians *Symplegma viride* is one of the least studied and the most interesting. It is typically West Indian in distribution, including southern Florida and Bermuda; it is also common in the East Indies and neighbouring waters.

A colony, in part, and a mature zoöid are shown in Fig. 1, together with a representation of the relationship of the buds in a growing margin of a colony. In size, thickness, and colors, and in size of constituent zoöids, *Symplegma* and *Botryllus* are much alike. They differ in some important respects. The zoöids in *Symplegma* are not arranged in coördinated groups or systems, and each zoöid has its own independent atrial siphon. Moreover, unlike *Botryllus,* the constituent zoöids of a colony of *Symplegma* persist while the colony lives and are not brief transients. As in *Botryllus,* vascular ampullae crowd the colony margins and exhibit rhythmical contraction. In a large colony little evidence of budding can be seen. In small and growing colonies, buds at various stages of development may be numerous in some regions of the colony margin. These may be seen in Fig. 1, *B.*

Mature zoöids are considerably larger than those newly formed by budding. Colonies, in fact, consist mainly of three types: small colonies with numerous buds and relatively small sexually immature active zooids; larger colonies with larger zoöids as yet sexually immature, and little sign of buds; and large colonies, again without buds, consisting of relatively large sexually mature zoöids. Each zoöid is connected with the common vascular system of the colony by a ventral stolon.

Apart from numerous differences in form and detail, the mature zoöid of *Symplegma* is markedly different from that of *Botryllus* with regard to gonads and buds. In *Symplegma* buds are not present on mature zoöids, while the gonad contains a maximum of about eight

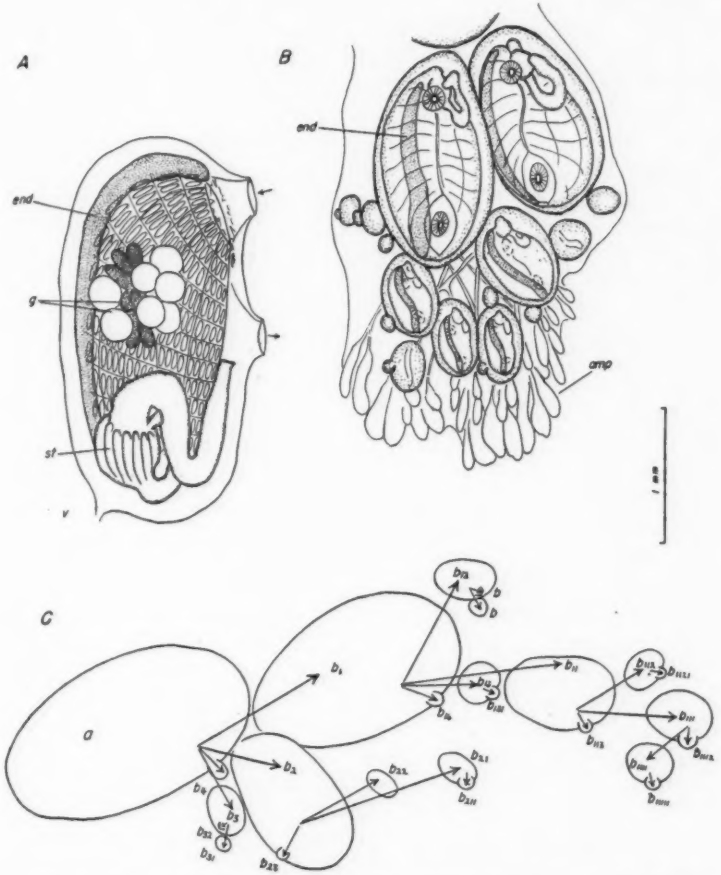

FIG. 1. Mature zoöid of a colony (*A*), and two camera lucida drawings of marginal growing zone of young colonies, at the same scale (*B* and *C*). The arrows in *C* indicate the origin, in sequence, of the buds produced in the course of development of each bud, all the buds shown having evolved directly or indirectly from the now active bud (*a*).

amp, vascular ampullae of colony; *end*, endostyle; *g*, gonad; *st*, stomach; *v*, ventral vessel connecting zoöid to colonial circulatory system.

large eggs as compared with the maximum of four characteristic of *Botryllus schlosseri.*

Development of the Egg

The fertilized egg develops in the atrial cavity of the parent and emerges as a swimming tadpole larva. The larva is typically botryllid in structure and activity, as has been emphasized by Caswell Grave (1932), although the degree of development of the permanent ascidian structure of the trunk region is relatively much less at this stage (cp. Berrill, 1935). The tadpole larva metamorphoses into a sessile zooid attached by eight ventral ampullae just as in *Botryllus.* It differs from the equivalent stage of the last form in that there are only four primitive gill slits (protostigmata) on each side of the pharynx. It also differs in that at this stage there is no sign of buds.

In any given generation of *Botryllus* zoöids there is no increase in number of rows of gill slits or number per row, once they have appeared in the developing zoöid. In *Symplegma,* on the other hand, the oozoöid grows continually in size, and correlated with this expansion (see Fig. 2) the primary gill slits subdivide into rows and in addition new subdividing stigmata successively appear in the ventral region of the pharyngeal sac. The general increase in the complexity of the branchial wall and the ampullary circulatory system external to the zoöid is shown in Fig. 2, *F.* At this stage two small buds have already appeared. These are shown at a larger scale in Fig. 2, *G.* The first of these has already lost its original attachment to the parent and has acquired its secondary connection by means of its ventral stolon with the ampullary circulatory system. The second and smaller bud is still attached by its wide stalk to the parental point of origin. In all of the several post-larval forms examined the relationships of the two buds at this stage were identical and there is no doubt that the buds all arise from a specific region of the body wall, namely on the right side near the base of the right ampullary vessel where it passes into the body wall. At this early stage therefore a rudimentary colony already exists.

The subsequent development of the colony can only be understood in the light of the development of the bud itself. It is already evident however, that compared with *Botryllus,* buds appear relatively belatedly, on one side of the body only, in series rather than as a single bud, and lose primary connection with the parent zoöid extremely early. The following section accordingly deals with bud development from a dual point of view, as a morphogenetic process and as a process of budding in relation to the developing colony.

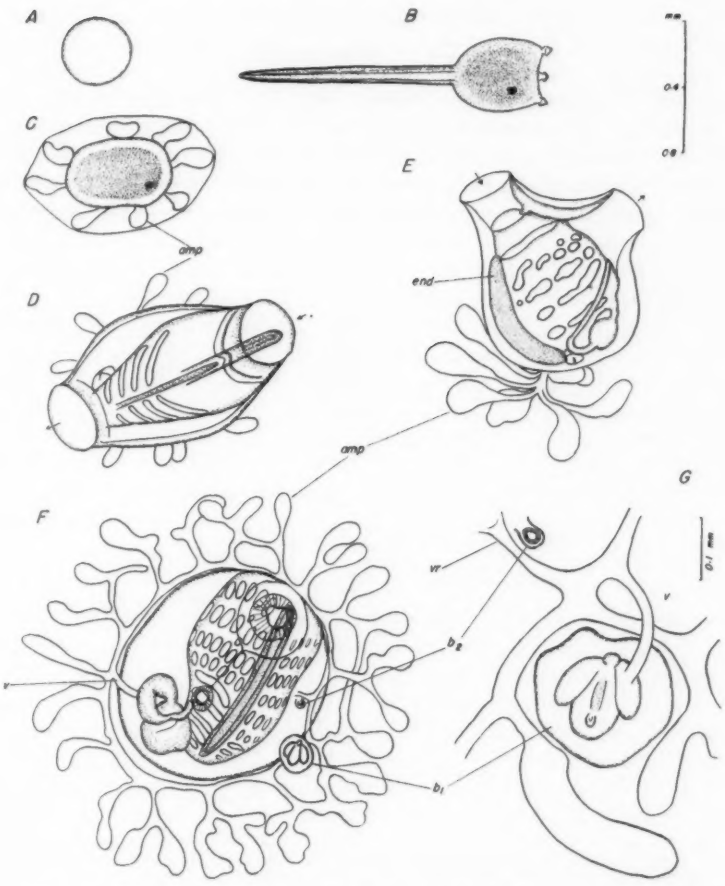

FIG. 2. Development from egg to first colonial organism. *A*, egg; *B*, swimming tadpole, showing three adhesive organs and single complex sense organ; *C*, metamorphosing stage with tail absorbed and eight vascular ampullae growing out as organs of attachment; *D*, first functional stage of ascidiozoöid shown in *C* with open siphons, and active gill splits in form of protostigmata; *E*, same individual as in *D*, after some growth, with protostigmata transforming into rows of definitive stigmata; *F*, later stage of same showing further subdivision of protostigmata, elaboration of ampullary system, and appearance of first two buds; *G*, the two buds and their relationship to the vascular ampullary system, at a higher magnification, the smaller still attached at its point of origin at the base of the right ampullary vessel, the larger having lost its original connection and acquired a secondary attachment through its own left ampullary vessel.

amp, ampulla; b_1, b_2, first and second buds; *end*, endostyle; *v*, left ampullary vessel; *vr*, right ampullary vessel.

Development of the Bud

Development of First-generation Buds

The development of the buds arising from the oözoöid (the zooid formed by the metamorphosed tadpole) can be described only partially. Three early stages of buds of the second series, and one later stage of buds of the first series, are available. These are shown in Fig. 3, and in general represent the two stages of buds shown in

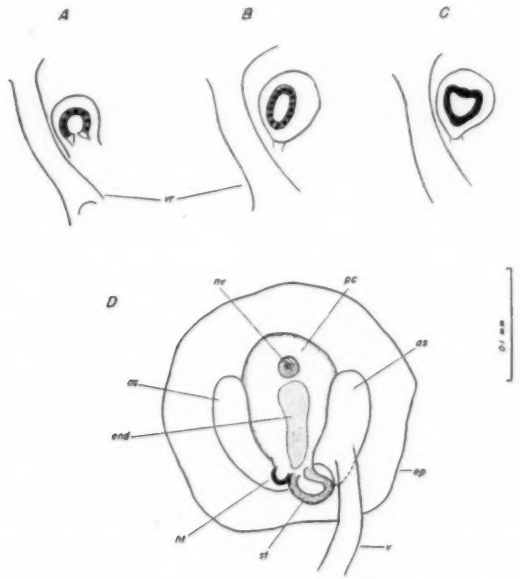

Fig. 3. Buds borne by primary individuals (oözoöid) of stage shown in Fig. 2, *F*.

A, B, C, three stages of second bud. *D,* first bud.

as, atrial sac; *end,* endostyle; *ep,* epidermis; *ht,* heart; *nv,* neural vesicle; *pc,* pharyngeal chamber; *st,* stomach; *v,* left ampullary vessel secondarily connecting bud with ampullary system of parent; *vr,* right ampullary vessel of parent near base of which all buds arise.

Fig. 2, *F* and *G*. The three smaller examples are shown in relation to the base of the right ampullary vessel, indicating the constancy in point of origin. In the smallest bud of the three the primary vesicle is not quite closed and the bud as a whole is united with the parental tissue by a wide stalk. The outer layer of the bud is continuous with the parental

epidermis and the inner layer with the atrial epithelium, just as in *Botryllus*. In the next largest bud the inner vesicle is a closed hollow sphere, while the outer layer is almost so, being attached to the parent by only a very narrow stalk. The stalk is still present in the third bud of the small series, but the inner vesicle is not only a little larger but already shows indication of subdivision into the essential organization units. The pair of folds that subdivide the chamber into the central pharyngeal and lateral atrial divisions can be seen just formed at the anterior margin, while a posterior thickening of the wall foreshadows the outgrowth of the intestinal region. In the later stage shown in Fig. 3, *D* these divisions are completed, and the central chamber has become further differentiated. The posterior thickening visible in Fig. 3, *C* has formed an intestinal outgrowth; endostylar grooves are forming on the ventral wall of the main sac, while an anterior and a right-posterior diverticulum or evagination represent the developing neural mass and heart respectively. The original stalk of attachment has disappeared and the bud has acquired a secondary connection, with the parental (now colonial) vascular ampullary system by means of an outgrowth from its ventral endostylar region.

Development of Buds in Older Colonies

The preceding section concerned mainly the origin and early development of the buds resulting in the conversion of the original individual developed from the fertilized egg into a real, though minute, colonial organism. Further details of bud development have been obtained only from the study of buds in larger and older colonies. The early stages of these are shown in Fig. 4. There is one significant difference between the following account and the one just given, quite apart from extension. The buds formed by the oözoöid arise from a fully differentiated and functional individual. The buds to be described arise from developing buds that are far from having attained the functionally differentiated state. In Fig. 4, therefore, each complete stage represents two bud generation stages and the relationship between them.

In Fig. 4, *A* the bud borne by the larger bud is at the stage of a disc in the process of becoming a hemisphere. In Fig. 4, *B* and *C* this process is continued until a hollow sphere, of atrial origin, is constricted off. A residual part of the original disc remains posteriorly in its original position.

In Fig. 4, *D* the epidermis enveloping the atrial vesicle is still widely connected with the parental epidermis. At the same time, in spite of

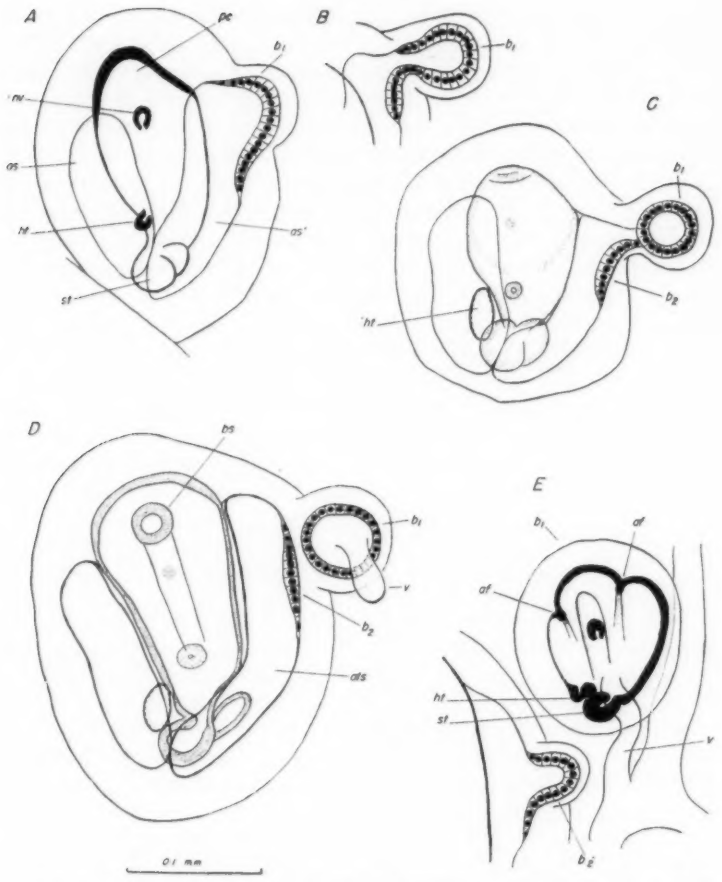

FIG. 4. Development of buds of advanced colony, as illustrated in Fig. 1, *B*.

A, a bud at approximately same stage as in Fig. 3, *D*, bearing in turn a bud of the succeeding generation, arising as a convex thickening disc of the right anterior atrial wall.

B, *C*, later stages in formation of bud shown appearing in *A*, showing formation of closed vesicle and residual area of disc.

D, three stages, a development of the larger zoöid shown in *A*, its first bud now a closed vesicle with a developing ampullary vessel, and a residual disc representing the first stage in bud development.

E, more advanced stage, with residual disc forming second vesicle and further residual area; first bud has acquired attachment to colonial circulatory system by its ampullary vessel and is completely separated from its parental bud.

af, atrial folds; *as*, atrial sac; *ats*, atrial siphon; b_1, b_2, first and second buds arising from larger bud shown in *A*; *bs*, branchial siphon; *ht*, heart; *nv*, neural vesicle; *st*, stomach; *pc*, pharyngeal chamber; *v*, ampullary vessel.

the fact that the inner vesicle is still a simple hollow sphere, the epidermis opposite one side of it has grown out as a vascular ampulla. This, as may be seen in the next stage (Fig. 4, *E*) joins the vascular

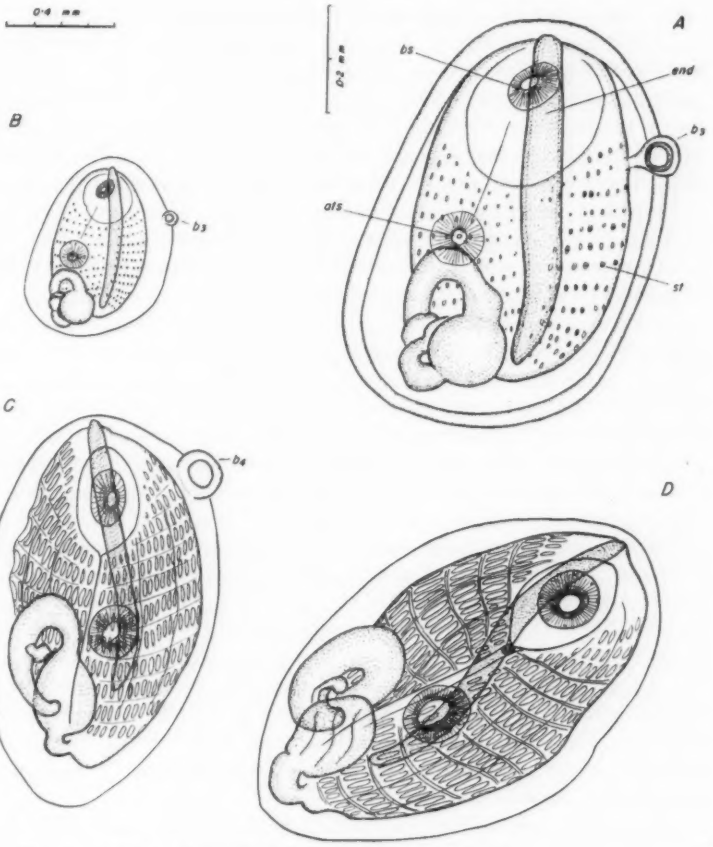

FIG. 5. Later development of bud up to fully active stage.

A, stage with rows of newly perforate stigmata, with third bud of series nearly separated. *B*, same stage shown at the reduced magnification of the succeeding stages. *C*, later stage giving rise to fourth and last bud. *D*, active stage complete except for absence of gonads, with no sign of further bud production.

circulatory system of the colony, and during the same period the bud is completely constricted off from the parent, epidermally as well as atrially. At this stage the main divisions of the organization are already

visible. The primary vesicle is being divided into the central and two lateral chambers by a pair of folds progressing antero-posteriorly. The diverticula or pouches representing the neural mass, heart, and intestine respectively have all appeared, and the endostylar limits are also visible. Later stages are shown in the parent buds illustrated in Fig. 4, *A*, *C*, and *D*. They are considerably larger but have not increased markedly in morphological differentiation. At the same time the process of budding exhibits some important features. The residual part of the disc giving rise to the buds described above becomes constricted off from the main part of the disc, as may be seen in Fig. 4, *B*, *C*, and *D*. This residual disc tissue grows somewhat in correlation with the growth of the parent bud and later transforms into a hemisphere, as in Fig. 4, *E*. This becomes a sphere and separates off to form a second independent bud just as in the first case, and again leaves behind a residual area of disc tissue in continuity with the atrial epithelium.

Continued development of the bud from the larger or parental bud shown in Fig. 4, *D* is to be seen in Fig. 5. Figure 5, *A* is drawn to one-half the scale of Fig. 4, and for spatial reasons the later stages are drawn to one-quarter of that scale. For purposes of comparison Fig. 4, *B* represents on the lesser scale the same stage shown in Fig. 4, *A*. At this stage much more morphological detail is apparent. The stomach and intestine are well differentiated, the siphons clearly marked, and the rows of numerous stigmata already perforate. A third bud, from the second residual disc area, is shown on the point of constricting off from the parent. In Fig. 5, *C*, at a stage where growth to the functional or active condition is almost reached, a fourth bud arising from the third residual disc area is to be seen constricting off. As far as can be determined, this represents the last of the bud series. In Fig. 5, *D* is shown the bud when it first attains the functional state, with open siphons and beating cilia in the gill slits. There is no sign of any further residual bud disc tissue. At this stage histodifferentiation is virtually completed, and the process of budding is to be associated with the developmental phase of the parent bud.

No sign of gonads has been detected in the newly functional zoöids. They develop during the subsequent active phase and mature in zoöids that are from two to three times in linear dimension the size they are when just active.

In Fig. 1 is shown in outline the zoöids in a marginal actively budding region of a young colony, showing the twenty-four buds arising directly or indirectly from zoöid *A*. The relationships are shown by the arrows.

Conclusions and Summary

As in *Botryllus,* the development of *Symplegma* is primarily the development of a colony and the development of an individual zoöid is modified accordingly.

Each developing zoöid or bud in the course of its development gives off a series of four buds from the anterior wall of the right atrial chamber. Individual zoöids when first active are less than one-half the final size and do not possess visible gonads. These appear later during the active or functional phase of growth.

As each bud disc develops into the vesicle stage, a residual part of the disc is unincorporated and remains to give rise to the next bud of the series, after a short period of growth. The production of buds thus comes to an end when growth of the parental organism as a whole is more or less complete.

The development of a bud, apart from the production of further buds, is an extremely direct process. A disc transforms into a hollow sphere. Two folds divide the sphere into a central (pharyngeal) and two lateral (atrial) chambers. Three pouches push out of the central division and give rise to heart, neural mass, and intestine. Later development is essentially an elaboration of these unit regions. Gill slits appear only when the pharyngeal wall has grown to a size permitting the simultaneous appearance, in miniature, of *all* the gill slits.

Buds, at a very early stage, lose their original connection with the parent organism and acquire a connection with the colonial circulatory system by means of a ventral stolonic vessel.

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OBSERVATIONS AND EXPERIMENTS ON THE PHYSIOLOGY OF MEDUSAE

LIBBIE H. HYMAN

(From the American Museum of Natural History, New York City)

Since the classical work of Romanes (1885) and the experiments of Morse (1906, 1907), Murbach (1903, 1907, 1909), Yerkes (1902, 1904, 1906), and Yerkes and Ayer (1903) on *Gonionemus,* there have been very few studies of the behavior and other general aspects of the physiology of hydroid medusae. Gemmill (1919) gave an account of the flagellar currents of *Mericertum,* Harvey (1921) studied bioluminescence in several Puget Sound medusae, Weese and Townsend (1921) tested the reactions of *Aequorea* to pH, temperature, and salinity, and Milne (1938) and Edney (1939) have published some notes on the behavior of fresh-water medusae. During a stay at the Oceanographic Laboratory, Friday Harbor, Washington, in the summer of 1938, I was led by the great abundance of hydromedusae in those waters to make some observations upon several matters. My principal object was to study the course of food in the gastrovascular system, a matter on which, so far as I am aware, no exact knowledge was available. Some other points were also investigated.

As material, five common Puget Sound medusae were utilized: *Aequorea aequorea, Halistaura cellularia, Phialidium gregarium, Stomotoca atra,* and *Sarsia mirabilis.* These species can be obtained near the dock in front of the laboratory buildings in great abundance at practically any time. Some observations were also made on *Gonionemus vertens* and the stalked scyphozoan *Haliclystus sanjuanensis.* The animals were studied immediately after being brought in from the Sound or in many cases were observed directly from the dock.

Water Content

Recently (1938), I presented some data on the water content of *Aurelia,* and reviewed the available literature. As nearly all the data concern scyphozoan medusae, it seemed desirable to obtain some figures on hydroid medusae. Accordingly, freshly collected specimens of *Aequorea, Halistaura, Phialidium,* and *Sarsia* were given a quick rinse in fresh water, dried on paper toweling, and weighed to the third place

in previously weighed beakers. They were then dried in an electric oven kept at 100° C., and the drying was completed to constant weight in a desiccator over $CaCl_2$. *Aequorea* and *Halistaura* are large species and were determined singly. In the case of the small species *Sarsia* and *Phialidium*, a number of specimens were used for each determination, as shown in Table I. The smallest available specimens of *Aequorea* were also determined for comparison with fully grown animals and two

TABLE I

Water content of Puget Sound hydroid medusae, arranged in order of decreasing weight. Salinity of sea water, 3.09 per cent.

Large Aequorea				Halistaura			
No. animals	Wet weight	Dry weight	Percentage water	No. animals	Wet weight	Dry weight	Percentage water
1	34.303	1.182	96.56	1	20.422	0.709	96.53
1	31.594	1.092	96.55	1	20.389	0.709	96.53
1	27.976	0.960	96.57	1	19.091	0.655	96.57
1	24.787	0.843	96.60	1	14.402	0.497	96.55
1	20.963	0.726	96.54	1	13.265	0.457	96.56
Small Aequorea				Sarsia			
2	8.669	0.296	96.59	5	2.350	0.080	96.60
3	12.647	0.387	96.94	6	2.549	0.090	96.47
2	6.395	0.204	96.81	22	8.343	0.269	96.78
3	8.555	0.258	96.99	7	2.459	0.085	96.55
3	8.522	0.273	96.80	18	2.838	0.090	96.83
2	5.667	0.194	96.58				
3	7.101	0.241	96.61	Phialidium			
3	6.346	0.205	96.77				
3	6.136	0.211	96.57				
3	4.589	0.134	97.08	12	4.923	0.165	96.65
				13	5.137	0.147	97.14
				18	6.975	0.243	96.52
				20	6.313	0.207	96.71
				15	4.558	0.157	96.56
				18	4.104	0.136	96.69

or three were weighed together in each case. The data are presented in Table I.

These data show that the water content of Puget Sound medusae ranges from 96.5 to 97 per cent and is therefore similar to that previously found for other medusae (Hyman, 1938), although perhaps slightly higher. The salinity at Puget Sound was stated to be 3.09 per cent, hence slightly lower than that at Mt. Desert Island, Maine, where

Aurelia was found to have a water content of around 96.2 per cent. As previously pointed out (Hyman, 1938), the water content of medusae depends on the salinity of the sea water in which they are living, but unfortunately the salinity is often not stated in the literature. It is, of course, possible that hydroid medusae have a slightly higher water content than scyphomedusae but without concomitant data on the salinity of the sea water this cannot be decided.

The data in Table I suggest that young *Aequorea* have a slightly higher water content than grown ones. Statistical analysis shows that the difference in the water content of the small compared with the large *Aequorea* is significant, being 3.54 times its standard error.[1] It may therefore be concluded that the water content of medusae declines with increasing age (size) and this result is consistent with what is known of the water content of other animals. The data also indicate that the small species (*Phialidium, Sarsia*) have a slightly higher water content than the large ones (*Aequorea, Halistaura*) but the difference was found to be without sufficient statistical significance.

Dr. Earl Norris, of the chemical staff of the Oceanographic Laboratory, permits me to quote the following analysis of *Aequorea* made in his laboratory: water content, 96.45 per cent, salts (ash) 2.70 per cent, and organic matter 0.85 per cent. The salt content thus appears to be less than that of the surrounding sea water and indicates some degree of control by the animal of the passage of materials through its surface.

General Behavior

The behavior of these medusae was found to be correlated in many respects with their morphology. *Stomotoca* and *Sarsia* are Anthomedusae with a tall, narrow bell, and long pendent manubrium. *Aequorea, Halistaura,* and *Phialidium* are Leptomedusae, with a broad flat bell and a very short manubrium. The bell is relatively thin and flexible in *Phialidium* and *Halistaura,* so that it contorts with each pulsation, especially in the latter, but *Aequorea* has an unusual amount of jelly, rendering the bell stiff and inflexible, except at the margin.

Observed from the dock, all the species showed the same general behavior. All pulsate at an even rate for a period of time, then cease pulsations, float in any position for a while, then resume pulsations; and this alternation of activity and quiescence continues indefinitely. As is well known, the rate of pulsation is more rapid, the smaller the animal. The periods of pulsation and quiescence appeared to have no absolute

[1] The statistical calculation was kindly made for me by Mr. Daniel Lehrman, using Fisher's t-formula.

or relative time value [2] except in *Stomotoca,* which regularly pulsates 3–8 times in succession and then pauses for an interval equal to 1–4 pulsations. The degree of extension of the tentacles bears no relation to the bell activity, i.e., the tentacles might be contracted or fully extended in either pulsating or floating specimens. It has been reported for some medusae (i.e., *Gonionemus,* Yerkes and Ayer, 1903) that when they touch the surface, they turn over and float downward in an inverted position. No trace of any such behavior was seen in any of the five species listed. Touching the surface has absolutely no effect upon them.

Reactions to touch were tested by a blunt glass rod, mostly on medusae in the water reachable from the dock. The exumbrellar surface is in general highly insensitive and a light to moderate touch usually elicits no response. A tap or blow on the exumbrella results, in all the

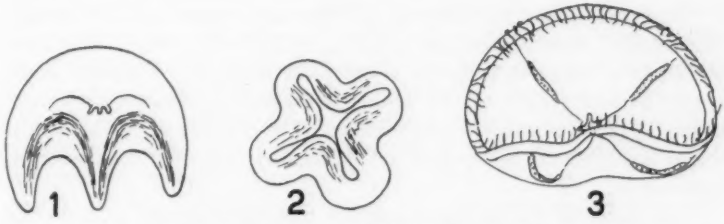

FIG. 1. Crumpling reaction of *Phialidium,* seen from the side.
FIG. 2. Crumpling reaction of *Phialidium,* seen from above.
FIG. 3. *Phialidium* giving the feeding reaction.

species except *Aequorea,* in a form of escape reaction which I call "crumpling." [3] The animal ceases pulsations, folds in the bell to the smallest possible compass (Figs. 1, 2), and sinks. The very stiff bell of *Aequorea* is incapable of such a reaction and, in fact, *Aequorea* gives no response even to severe blows which knock it for several inches, except that it may miss a pulsation. Bumping into objects often evokes the crumpling reaction.

Mechanical stimulation of the bell margin, if effective, evokes either an escape reaction or the feeding reaction. The escape response may consist of the crumpling behavior noted above or of bell pulsations. The sensitivity of the margin appeared to vary with species. In *Hali-*

[2] Romanes (1885, p. 147) has recorded the number of pulsations and the duration of the rest periods in seconds for three specimens of *Sarsia* and also found no correlation between the lengths of active and quiescent periods.

[3] What Romanes (1885, p. 123) called a "spasm" in *Staurophora,* "a sudden and violent contraction" causing the bell to assume a cuboidal form, is probably the same response as here termed "crumpling."

staura even a light touch often induced crumpling, whereas in the other species, a light touch was often ineffective. In *Phialidium* and *Sarsia,* marginal stimulation of the floating animal often resulted in the resumption of pulsations. *Stomotoca* and *Aequorea* were relatively insensitive to marginal stimulation. Strong marginal stimulation commonly results in crumpling in all forms except *Aequorea.* This animal, as already noted, is anatomically incapable of this type of escape reaction; to marginal stimulation, it either responds not at all or gives the feeding reaction, described below. Marginal stimulation may cause a cessation of swimming in a pulsating animal (*Phialidium*); this may be a form of feeding response. In the anthomedusan forms (*Sarsia, Stomotoca*), the manubrium, which hangs down below the level of the bell margin, appeared to be much more sensitive than the latter and would respond by crumpling or the feeding reaction to an intensity of touch which failed to evoke any response on the margin.

Response to light has been recorded for some medusae (*Gonionemus,* Morse, 1907; *Sarsia,* Romanes, 1885). In the forms here observed not the slightest response could be noted when they passed from a sunlit region into the shadow of the dock nor was any tendency ever seen to collect in either shaded or sunlit areas. The distribution of these medusae appeared to be determined wholly by the tidal currents and movements.

The water of Puget Sound near the laboratory is very cold, around 10° C. Any rise of more than a few degrees above this temperature diminishes the irritability of the medusae. At temperatures of 15–18° C., they tend to lie upon the bottom of the vessel in a flaccid, unresponsive condition, although pulsations continue. Weese and Townsend record that pulsations cease at 21–23° C.

Some medusae exhibit a food-catching ("fishing") behavior, swimming to the surface, then floating down, either right side up or inverted, with tentacles fully extended (*Gonionemus,* Yerkes and Ayer, 1903; *Limnocnida,* Edney, 1939). No food-catching behavior of any sort was witnessed in the Puget Sound medusae. They appear to depend wholly on chance contacts with suitable animals.

In the presence of food (giving off juices) *Gonionemus* shows excitement and makes random "searching" movements. The Puget Sound medusae, when placed in a vessel with a crushed bit of animal flesh, generally gave not the slightest sign of response even when quite close to the food. Occasionally, however, *Phialidium* when near the food would "hover" for a brief period. It seems probable that these medusae are capable of perceiving animal juice in the water.

The Feeding Reaction

To mechanical or chemical stimulation of the bell margin, all the species observed may give the feeding reaction. Mechanical stimulation consisted in touching the bell margin with a glass rod or forceps or moving the rod or forceps for a short distance along the margin. As already noted, such stimulation often evokes no response or may cause an escape reaction. Chemical stimulation consisted in touching a bit of animal flesh held in a forceps to the margin. As sources of food, fish, barnacles, *Mytilus,* crabs, etc., were used. The tentacles practically always grasp food and may grasp an inert object; the feeding reaction nearly always follows grasping of food by the tentacles, but does not invariably occur. If the feeding reaction fails to take place, the tentacles drop the food after a time.

The nature of the feeding reaction is correlated with the morphology of the animal. In the Leptomedusae (*Aequorea, Halistaura, Phialidium*) and also *Gonionemus* (Yerkes, 1902) with broad shallow bells, the reaction consists in the bending of the stimulated part of the margin to the manubrium (Fig. 3). The manubrium, which is very short in these forms, also moves to meet the inbent margin. The margin is held against the manubrium for a period during which the manubrium usually, but not invariably, grasps the food. When this has occurred, the margin returns to its normal position. If the manubrium fails to attach to the food, the margin will usually drop the food after a time. An *Aequorea* was observed in which the margin with food attached repeatedly bent to touch the manubrium until finally the manubrium grasped the food.

The following experiments were performed on *Phialidium,* which proved the most suitable form for studying the feeding reaction. If while one part of the margin is performing the feeding reaction, i.e., is being held against the manubrium, another part of the margin is stimulated, it too will give the reaction, providing the interval between the two stimulations is not too short. A third point of the margin may also be induced to respond; although stimulation of a third point may instead release another feeding reaction from the first region stimulated. The fact that the animals often fail to respond makes it difficult to study these reactions in detail or to investigate the question of fatigue.

The feeding reaction in the Anthomedusae (*Sarsia, Stomotoca*) differs from that of the Leptomedusae. These animals have a tall narrow bell and a long manubrium hanging below the level of the bell margin (Figs. 4, 5). The feeding reaction consists in the bending of the manubrium towards the stimulated part of the margin. The margin

does not show any movement. The manubrium was not seen to touch the margin but simply to make a nearly right-angled bend towards the stimulated region. In case of food held by the tentacles, the manubrial lip would touch and grasp the food.

In a leptomedusa which he called *Tiaropsis indicans* (stated by Mayer, 1910, to be probably *Eutimium socialis*), Romanes (1885) found that the tip of the manubrium would be precisely applied to any strongly stimulated spot of the bell. No such exact reactions were seen in any of the Puget Sound medusae.

In all the species observed, the lip of the manubrium (mouth frill) was found to be far more sensitive to chemical stimulation than any other part of the body. Food touched to the mouth frill was invariably

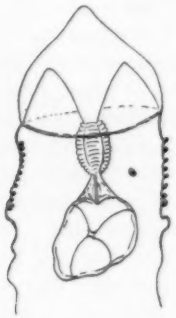

FIG. 4. *Stomotoca* with mouth frill attached to a *Phialidium*.

grasped. Probably the mouth frill in forms with a long manubrium is the chief agent in food capture.

Very few of the numerous medusae observed around the laboratory docks were seen to contain ingested food. It seems probable that the food requirements of these animals are low. *Sarsia* was never seen with ingested food. *Stomotoca* regularly attacks other medusae and was not infrequently seen with the expanded mouth attached to *Phialidium* (Fig. 4), sometimes to the larger forms. *Stomotoca* was also observed attempting to capture a crustacean larva, which escaped. *Phialidium* appeared to feed chiefly on small or minute plankters but might ingest larger crustacean larvae. *Aequorea* appeared to be a somewhat indiscriminate feeder and was observed with ingested crustaceans, *Nereis*, and peas and salmon refuse from adjacent canneries. Following a night run of a small *Nereis*, *Aequorea* and *Halistaura* would be seen on the next morning with ingested *Nereis*.

Course of Food in the Gastrovascular System

As already mentioned, the chief purpose of these observations was to study the function of the gastrovascular system. To my knowledge, no proof exists that food is actually carried along the canals of this system, although statements to this effect are standard in textbooks. To study the matter, bits of fish, barnacles, *Mytilus,* or crabs were dipped into a thick paste made of powdered carmine and sea water and then touched to the mouth frill of medusae. Such bits were invariably grasped and usually, although not always, swallowed in a short time. *Stomotoca* and *Sarsia* proved rather refractory subjects and would often fail to ingest the piece of flesh. The course of the ingested material is easily followed by means of the red carmine.

Within half an hour after ingestion red material is seen spreading along the radial canals and within two or three hours it occupies the entire gastrovascular system. The material appears to be driven along the canals chiefly by the bell contractions. At each contraction, the material moves back and forth or swirls about. Gemmill (1919) in *Melicertidium* (correct name, *Melicertum*) found definite flagellar currents, running peripherally along the roof, centrally along the floor of the radial canals. Examination of Puget Sound medusae under the microscope failed to show any definite currents in the gastrovascular canals, although the flagella could be seen in active motion.

Immediately the food reaches any region of the gastrovascular system it is subject to ingestion by the gastrodermis,[4] where it undergoes intracellular digestion. Surprisingly enough, however, the rôle of the gastrodermis in intracellular digestion varies greatly throughout the gastrovascular system. The chief sites of intracellular digestion are the gastrodermis of the manubrium and stomach (cavity at the upper end of the manubrium) and of the tentacular bulbs (basal swellings of the tentacles). These regions soon become stained deeply pink from ingested carmine particles (Figs. 5, 6, 7). Radial, circular, and tentacular canals take up some particles but play a minor rôle in comparison to manubrium, stomach, and tentacular bulbs. In forms such as the Leptomedusae which bear the gonads on the radial canals, those portions of the radial canals adjacent to gonad tissue were good sites of intracellular digestion; in non-gonadal regions the radial canals ingested very few carmine particles (Figs. 6, 7). In *Sarsia,* an expanded region near the manubrial tip is termed the stomach and is, in fact, the place where the ingested food is held, whereas in most medusae the stomach is an

[4] I have elsewhere introduced the term *gastrodermis* for the inner epithelium of coelenterates, usually called entoderm, in the belief that the terms ectoderm and entoderm had best be restricted to embryonic stages.

expanded region in the summit of the bell, where the radial canals originate, and the food is passed into this chamber although, if too large, part of it remains in the lumen of the manubrium. In *Sarsia* it was found that the entire manubrium has as good digestive powers as the so-called stomach region (Fig. 5) and this was also the case with the other medusae. In short, the gastrodermis not only of the stomach but of the whole manubrium has high powers of intracellular digestion, equalled only by the gastrodermis of the tentacular bulbs. Sections through the tentacular bulbs of medusae showed that the gastrodermis is here highly columnar and packed with food vacuoles.

It is well known that in some hydroid medusae the radial canals open to the exterior by a pore near the bell margin (Fig. 7). These pores have been presumed to be excretory. In *Aequorea,* which possesses such pores, strands of mucus with entangled carmine particles were seen exuding from these pores a couple of hours after carmine feeding. This observation indicates that the pores may serve for the ejection of indigestible material.

Carmine particles persist in the gastrodermis for at least two or three days after feeding. The fed medusae were not retained long enough to determine the time interval required for complete elimination of the fed carmine.

Gastric Juice of Aequorea

Following the example of Beutler (1924), gastric juice was collected from *Aequorea* by feeding the animals small bits of sponge soaked in animal juice. Since it was soon learned that the juice of *Mytilus,* barnacles, etc., contains enzymes, all such juice was thereafter boiled. Small bits of boiled bath sponge were soaked in such boiled juice and then touched to the mouth frill of *Aequorea,* which always grasped them. Ingestion usually followed, but not infrequently the piece of sponge would be soon ejected. The experiments were not as satisfactory as hoped, because even when retained, the piece of sponge was seldom taken thoroughly into the gastric cavity, partly because of the incompressibility of the sponge and partly because of the stiff thick jelly of *Aequorea.* However, in many cases the sponges were retained in the manubrium for a considerable period. The pieces of sponge were then withdrawn with a forceps and their contained fluid expressed into a beaker. Some simple tests for enzymes were made with this fluid on litmus milk, bits of boiled animal flesh, and starch solution. The presence of a protease capable of dissolving animal flesh and of a lipase were indicated but amylase appeared to be absent.

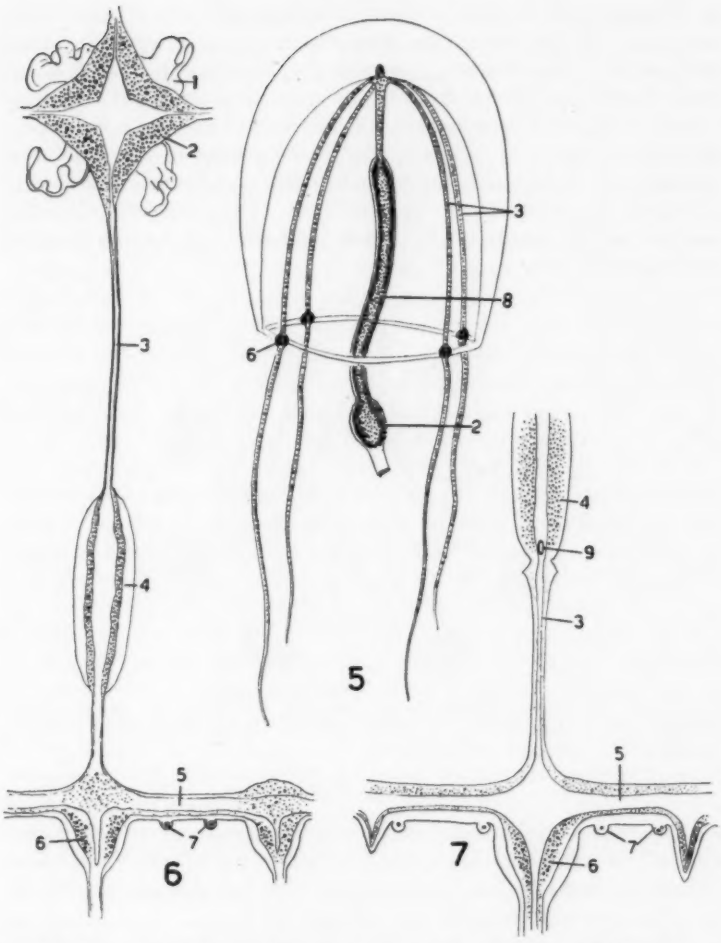

FIG. 5. *Sarsia*, following ingestion of carmine, showing distribution of carmine in the gastrodermis.

FIG. 6. *Phialidium*, stomach, one radial canal, and piece of margin, showing distribution of carmine in the gastrodermis, six hours after ingestion.

FIG. 7. *Aequorea*, piece of margin and adjacent part of one radial canal, showing distribution of carmine in the gastrodermis. Abbreviations: *1*, mouth frill, *2*, stomach, *3*, radial canal, *4*, gonadal region of radial canal, *5*, circular canal, *6*, tentacular bulb, *7*, statocysts, *8*, manubrium, *9*, so-called excretory pore.

A number of *Aequorea* containing an ingested prey, chiefly *Nereis*, were captured and the gastric fluid removed by a fine pipette. Such fluid was in all cases but one more acid than the sea water, which has in Puget Sound a pH of 8.0–8.2. Of 12 individuals, the gastric juice was 7.2 in 1 case, 7.4–7.6 in 9 cases, 7.8 in 2 cases, and 8.0 in the twelfth specimen. A piece of *Mytilus* placed in the combined juice from four animals, with a pH of 7.4–7.6, was much dissolved in three hours, while a control piece in sea water was unaffected. The possibility, of course, remains that the acidity of the gastric juice might have come from the disintegrating prey.

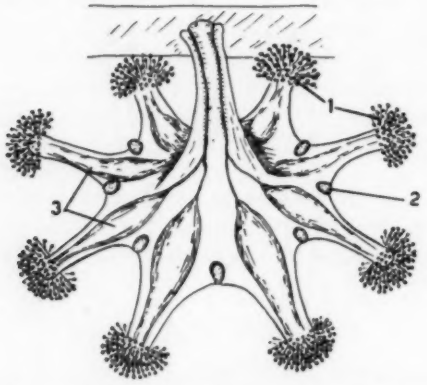

FIG. 8. *Haliclystus*, pendent from a seaweed, seen from the aboral surface, showing anchors and arms with tentacle clusters. *1*, tentacle cluster, *2*, anchor, *3*, gonads.

BEHAVIOR OF HALICLYSTUS

The stalked scyphozoan, *Haliclystus sanjuanensis*, one of the Stauromedusae, is common on seaweeds in a shallow bay of San Juan Island. During exceptionally low tides one can wade out and observe this interesting form in its natural surroundings.

Haliclystus (Fig. 8) has a trumpet-shaped body with the flaring oral end scalloped into eight arms, each terminating in a ball of small capitate tentacles. In the bottom of each scallop is an oval body, called the anchor, which, according to Schlater (1891), is provided with sensory cells and mucous glands. Seen in their natural habitat, the animals are fastened by the pedal disk to seaweeds and hang in a pendent attitude with the anchors erected. The medusa is relatively inactive. The chief activities seen in nature were: swinging on the stalk, flicking of the tentacles, sudden bending of an arm to the manubrium, or the folding

of all arms against the manubrium. When touched with a glass rod, the tentacle clusters might not react but often grasped the rod tenaciously and executed the feeding reaction. This consists of the quick bending of an arm so that the tentacle cluster touches the manubrium. It is very quickly given when food is touched to the tentacle cluster and arms other than the one touched may join in the reaction. The chief food in nature appeared to be the amphipod *Caprella*, common on the seaweeds where *Haliclystus* lives.

Brought into the laboratory, *Haliclystus* does not remain in good condition very long. Attempts to feed the animals with carmine-coated *Caprella* failed. The tentacles quickly grasped the food and bent with it to the manubrium which accepted it; but ingestion never ensued and the food was dropped after a time. Detached *Haliclystus* made strenuous efforts to reattach themselves, using the tentacle clusters for adhesion, but never succeeded even when placed on seaweeds. The tentacle clusters are extensively used in adhering to objects. No function could be discovered for the anchors. They were utterly unresponsive to mechanical or chemical stimulation, even severe pushing about, and although always credited with adhesive powers, were never seen to be employed by the animal for this purpose. The tentacle clusters appeared to be the most sensitive part of the animal. When touched they adhere and usually give the feeding reaction. Mechanical stimulation of the manubrium was ineffective; touching the subumbrellar surface usually evoked the feeding reaction in the adjacent arm. Strong mechanical disturbance of the animal may result in the infolding of all the arms, a response resembling the crumpling reaction of free medusae. Flicking of the tentacles or bending of the arms toward the manubrium were often witnessed without apparent cause; perhaps microscopic food is being caught at such times.

The Function of the Tentacular Bulbs of Hydromedusae

The tentacle bases in hydroid medusae are commonly enlarged, forming the tentacular bulbs (Figs. 6, 7). The function of these bodies appears to have been frequently misunderstood. Because in some Anthomedusae each bulb bears an ocellus, the entire bulb is often called an ocellus in books and laboratory manuals. The bearing of an ocellus is, however, only one function of the tentacular bulbs and in fact not their usual function.

My study of the tentacular bulbs of a number of medusae has shown that the epidermis of these bulbs contains much sensory epithelium and is also a depot for the manufacture of nematocysts (these functions were already known). The epidermis is crowded with cnidoblasts in process

of secreting nematocysts and there can be no doubt that the tentacular bulbs furnish nematocysts for the rest of the body. I have also discovered, as already noted in the discussion of intracellular digestion, that the gastrodermis of the tentacular bulbs plays an important rôle in this process. This gastrodermis is a tall columnar epithelium which after food ingestion becomes packed with food vacuoles. The intense colors often seen in the tentacular bulbs and frequently carefully described in taxonomic accounts represent food materials in the gastrodermis of the bulbs and hence are of no systematic value.

In *Gonionemus* and other trachymedusae, the tentacular bulbs are often very large and conspicuous and partially or wholly detached from the tentacle bases. The tentacular bulbs of *Gonionemus murbachii*, found in the Eel Pond at Woods Hole, contain a black pigment and this has led to the erroneous idea that these bulbs are ocelli or "eye-spots." Thus in the last edition of Drew's "Invertebrate Zoology," it is stated that these bulbs are probably photosensitive, despite the fact that Murbach (1907) failed to find any evidence that they are more sensitive to light than other parts of the bell. I have not had any specimens of *G. murbachii* available for sectioning but I have sectioned the tentacular bulbs of *G. vertens*, the Puget Sound species, fixed in Flemming's fluid for the purpose. The sections show no trace of a photoreceptor in the bulbs of this species although there is an abundance of sensory epithelium. In fact, photoreceptors are not known to occur in any of the Olindiidae, the family to which *Gonionemus* belongs. I think it may safely be concluded that the species of *Gonionemus* are devoid of special photoreceptors although these medusae do react to light.

The tentacular bulbs of hydromedusae thus have three important functions: they act as depots for the manufacture and storage of nematocysts, they are organs of general sensory perception, and they are very active in intracellular digestion.

Summary

1. Studies were made in Puget Sound on several medusae belonging to the groups Anthomedusae (*Stomotoca, Sarsia*), Leptomedusae (*Aequorea, Halistaura, Phialidium*), and Stauromedusae (*Haliclystus*).

2. The water content was determined for four species (*Aequorea, Halistaura, Phialidium, Sarsia*) and found to range from 96.5 to 97 per cent (salinity of the sea water 3.09 per cent). Evidence was obtained that the water content declines slightly with increasing size.

3. Activities are limited and responses cannot be elicited with dependable regularity.

4. All species observed have alternate periods of pulsation and floating; in general these periods bear no absolute or relative time duration to each other.

5. Behavior is correlated with morphology and hence shows characteristic differences between the different groups.

6. Mechanical or chemical stimulation, when effective, elicits either an escape reaction or the feeding reaction.

7. The escape reaction may consist of bell pulsations but to stronger stimuli a general contraction, termed crumpling, accompanied by cessation of swimming, is given. *Aequorea* is anatomically incapable of this escape reaction because of its thick stiff bell.

8. The feeding reaction consists in the Leptomedusae and *Haliclystus* of bending the stimulated region of the margin to the manubrium which also moves to meet the inbent margin. In the Anthomedusae it consists of turning the long manubrium in the direction of stimulation.

9. Food was traced through the gastrovascular system by feeding bits of animal flesh coated with carmine. Within half an hour after feeding, food begins to spread along the radial canals and in two or three hours occupies the entire gastrovascular system.

10. The principal sites of intracellular digestion are the lining epithelium of the manubrium, stomach, and tentacular bulbs. Radial, circular, and tentacular canals play a minor rôle. Those portions of the radial canals adjacent to gonads (Leptomedusae) are much more active in intracellular digestion than radial canals elsewhere.

11. No definitely directed flagellar currents could be observed in the gastrovascular system.

12. Strands of carmine-containing mucus were seen to be extruded from the so-called excretory pores of the radial canals (*Aequorea*).

13. The fluid from the stomach of *Aequorea* is usually considerably more acid than sea water (to pH 7.2, mostly 7.4–7.6). It contains a proteolytic and a lipolytic enzyme but appeared to be devoid of diastases.

14. Feeding and escape reactions of *Haliclystus* (a sessile stauromedusan) are similar to those of Leptomedusae. No function could be found for the anchors; the animal gave no response to chemical or mechanical stimulation of the anchors nor were they used for adhesion.

15. The tentacular bulbs of hydromedusae function as depots for the manufacture and storage of nematocysts, as organs of general sensory perception, and as important sites of intracellular digestion. They are not photoreceptors although in Anthomedusae they bear the ocelli when these are present. The tentacular bulbs of *Gonionemus* are devoid of differentiated photoreceptors.

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THE ANAEROBIC GASEOUS METABOLISM OF THE ROACH, CRYPTOCERCUS PUNCTULATUS SCUDDER

DARCY GILMOUR [1]

(From the Biological Laboratories, Harvard University, Cambridge, Mass.)

In the experiments of Davis and Slater (1926, 1928) on the anaerobic metabolism of the cockroach, *Periplaneta orientalis*, the oxygen debt incurred during anaerobiosis was paid off exactly, and the excess respiration of recovery appeared to be due to the complete oxidation of the lactic acid produced. I have recently shown (Gilmour, 1940) that in the termite, *Zootermopsis nevadensis*, about half the oxygen debt is repaid, and that the carbon dioxide produced during anaerobiosis is not all due to the buffering of acids.

The general habits and mode of nutrition of the primitive wood-feeding cockroach, *Cryptocercus punctulatus* Scudder, are similar in many ways to those of the lower termites. In common with them, the roach possesses a well-developed intestinal fauna of flagellates, which are responsible for the digestion of cellulose (Cleveland, 1934). It seemed likely, therefore, that a consideration of the anaerobic metabolism of this insect, which in some ways seems to occupy a position intermediate between the higher roaches and the more primitive termites, might yield information on the nature of the differences in the anaerobic processes of the two groups.

Acknowledgments

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Material and Methods

The insects were collected near Blacksburg, Virginia. Two different batches of material were used in the experiments. The first was collected in the fall of 1938 and kept in the laboratory during winter

[1] James King of Irrawang Scholar of the University of Sydney.

at a room temperature of about 22° C.; the second was collected in the spring of 1939 and kept during the summer in a constant-temperature room at 15° C. The diet consisted of moist paper towelling or filter paper.

Respiration was measured by means of Warburg manometers. Two different types of respirometer vessel were used. The first was that described by Pomerat and Zarrow (1937); the other was of the common conical design without centre-well and with a double side-arm. Respiratory quotients were measured by following oxygen uptake for a certain length of time (usually one hour) and then determining the carbon dioxide absorbed, by acidification of the alkali. Fully-grown roaches were studied throughout, one insect being used in each experiment.

Activity was reduced to a minimum by lowering the temperature and placing each insect in a small cage. The cages were made of perforated celluloid, the perforations being sufficiently large and numerous that there was no interference with the diffusion, in and out, of gases. The temperature of the experiments in which the first batch of animals was investigated was 7.5° C. For the second batch, it was necessary to reduce the temperature to 5° C. in order to eliminate all movement. This difference may have been due to the fact that the temperature at which the insects were cultured was lower in the second group than it had been in the first.

Anaerobic conditions were produced by running pure nitrogen (moist and equilibrated to the temperature of the water bath) through the respirometer vessel for a period of 15 to 20 minutes. At the end of the anaerobic period the nitrogen was replaced by CO_2-free air.

The insects were defaunated by exposing them to oxygen at 4 atmospheres pressure for 2 hours.

Experimental Results

Normal Insects

Aerobic Respiration.—The oxygen consumption of normal individuals was 46.1 cu. mm. per gram wet weight per hour at 7.5° C. (average of 70 experiments varying between 23.8 and 68.5), and 28.5 cu. mm. per gram per hour at 5° C. (average of 12 experiments varying between 20.5 and 37.5).

The respiratory quotient varied widely (0.79–1.65), the average of 30 experiments being 1.09. There were no significant differences between the results of experiments run at 5° C. and those at 7.5° C.

The production of combustible gases by the protozoa was investigated in a number of experiments, in which the insects were placed in a closed chamber and the gases present after an interval of some hours analysed with a Haldane apparatus. The protozoa of the roach were found to produce much smaller quantities of combustible gases than did those of the termite. The rate of evolution at 22° C. was about 2 cu. mm. per gram insect per hour—less than 1 per cent of the insect's oxygen uptake, whereas in the termite it has been shown to be about 10 per cent. The analyses indicated that hydrogen was not the only gas evolved, since in almost every case some carbon dioxide was formed as a result of the combustion. It was not possible, however, to arrive at any quantitative estimation of the amounts of different fractions present, since, even when the experiments were continued for such a long time that a large proportion of the oxygen present had been used up, the concentration of combustible gases in the sample analysed was usually less than 0.1 per cent.

This slow rate of evolution of combustible gases was confirmed subsequently in the anaerobic experiments, there being no significant increase in pressure when the respirometers were filled with nitrogen and carbon dioxide was being absorbed by alkali.

The high respiratory quotient is most probably due here, as in the termite, to the presence of large numbers of anaerobic protozoa.

Effects of Anaerobiosis.—When returned to air after 4 hours in nitrogen at room temperature, *Cryptocercus* remained motionless for 1 to 1½ hours, and thereafter showed increasing activity, returning apparently to normal after about three hours. An anaerobic period of 5 hours proved fatal to some, while others recovered, but none survived 6 hours in nitrogen. There was a partial recovery after 6 hours, and even to a smaller extent after 7 hours, but the insects were all dead on the day following the treatment.

Oxygen Debt.—In all the oxygen debt experiments the oxygen consumption was first followed for 2 to 3 hours, after which the air in the respirometers was replaced by nitrogen. After an anaerobic period of 1 hour, the recovery respiration was followed until a steady rate of oxygen consumption had been re-established and maintained for 2 or more hours. The results of these experiments are presented in Table I. The final rate of oxygen uptake is not far removed from the initial rate, but there is, in general, a tendency for the rate after recovery to be higher. The final rate, however, seems to be quite as constant as the initial, and has been followed for as long as 4 hours.

Cryptocercus constantly consumes during recovery an excess amount of oxygen greater than the amount of oxygen missed in anaerobiosis,

and, in fact, repays the debt, on the average, three times over. By " oxygen missed in anaerobiosis " is meant the amount of oxygen which the insect would have consumed during the same period if it had been in air. The time required to effect complete recovery after only 1 hour of anaerobiosis was 4 hours.

Carbon Dioxide Excretion During and After Anaerobiosis.—Experiments on carbon dioxide excretion during anaerobiosis were performed in the same way as for a determination of respiratory quotient,

TABLE I

Repayment of oxygen debt in untreated insects.

The duration of anaerobiosis was approximately one hour in each case. Any slight deviations from this time have been taken into account in calculating the oxygen missed.

Experiment	Temperature °C.	Initial O_2 uptake $mm^3./gm./hr.$	O_2 missed $mm^3./gm.$	Final O_2 uptake $mm^3./gm./hr.$	Excess O_2 consumed $mm^3./gm.$	$\frac{\text{Excess } O_2 \text{ consumed}}{O_2 \text{ missed}} \times 100$
A 16 V	7.5	50	58	55	187	320
A 16 IV	"	43	50	55	133	270
A 25 I	"	41	48	47	142	300
A 25 IV	"	41	48	54	133	280
A 27 (2)	"	56	70	60	223	320
A 27 I	"	41	48	47	132	280
A 27 IV	"	39	46	52	95	210
A 27 V	"	41	48	56	191	400
A 27 (1)	"	40	50	43	167	330
B 1 (1)	"	57	57	69	106	190
B 1 (2)	"	52	52	38	91	180
Average		46	52	52	145	280
B 2 (i)	5.0	30	30	31	99	330
B 2 (ii)	"	27	27	29	79	290
B 2 (1)	"	23	23	28	80	350
B 2 (2)	"	26	26	29	112	430
B 32 (2)	"	34	34	38	123	360
Average		28	28	31	99	350

except that the air in the respirometer was replaced by a rapid stream of nitrogen before the run was started. The carbon dioxide taken up by the alkali was liberated after an anaerobic period of 1 hour in each case. The results of 4 experiments, all done at 5° C. were as follows:

(cu. mm. per gram) 79 89 78 75 average 80.

The amount of carbon dioxide excreted during anaerobiosis is thus almost three times as great as the amount of carbon dioxide produced during a similar period in air.

TABLE II

Respiratory quotients at successive one-hour periods after anaerobiosis. Untreated insects.

These figures do not represent successive determinations on single insects. Each quotient is obtained from a separate individual.

Hourly periods	1	2	3	4	5	6
	0.31	0.58	0.86	0.90	1.00	1.02
	0.34	0.80	0.84	1.00	0.82	0.92
	0.71	0.78	0.80	0.55	1.18	1.17
	0.51	0.52	0.45	0.55	1.30	0.98
R.Q.	0.46	1.12	1.04	1.05	0.99	1.14
	0.58	0.54	0.77	1.13	1.14	1.00
	0.57	0.75	0.86	0.92	0.97	1.13
	0.55	0.67	0.65	0.96	1.45	1.65
	0.59	0.92	0.97	0.76	1.18	—
	—	0.90	0.71	0.63	—	—
Average	0.51	0.76	0.80	0.85	1.11	1.13

The carbon dioxide retained during recovery was determined by subjecting the insects to nitrogen treatment for 1 hour, and then observing respiratory quotients over 1-hour periods for 6 successive hours. Since the individual variation in respiratory quotient is great, it was necessary to make many different determinations for each 1-hour period

TABLE III

Carbon dioxide retention during recovery. Untreated insects.

Hourly periods	O_2 consumed $mm^3./gm.$	CO_2 produced $mm^3./gm.$	Expected CO_2 production $mm^3./gm.$	CO_2 retained $mm^3./gm.$
1	65	33	69	36
2	72	55	76	21
3	47	38	51	13
4	45	38	49	11
5	31	35	35	—
6	31	35	35	—
			Total	81

in order to obtain significant mean values. The results of these experiments are given in Table II. There is a reduction in respiratory quotient during the recovery period. By the end of 4 hours the quotient has returned to its normal value.

In Table III is shown the carbon dioxide excretion for each hour, calculated from the respiratory quotients and the curve for average

oxygen consumption. The carbon dioxide retained is determined by subtracting the actual carbon dioxide production from the theoretical, the latter being calculated on the assumption that the recovery process consists of an excess respiration having a respiratory quotient of 1.0 superimposed upon the "basal" respiration. The total amount of carbon dioxide retained is 81 cu. mm. per gram, which agrees very closely with the amount of carbon dioxide excreted during anaerobiosis.

Defaunated Insects

Aerobic Respiration.—All experiments on defaunated roaches were carried out at 5° C.

The average oxygen consumption was 44.6 cu. mm. per gram per hour (28 experiments, with results varying between 31.0 and 65.8). There is thus a decided increase over the rate of respiration of untreated insects. A small fraction of this increase is presumably due to a decrease in weight by the removal of anaerobic protozoan tissue. The average weight of defaunated insects was 0.67 gram, that of untreated insects, 0.73 gram.

The average respiratory quotient following defaunation was 0.69 (16 experiments, varying between 0.35 and 0.99). The results, obtained at intervals between the first and fifteenth days following defaunation, showed no definite trend with time.

Oxygen Debt.—The course of oxygen consumption during recovery from anaerobiosis in defaunated roaches is by no means the same as in normal insects. In Table IV are collected the results of experiments on the oxygen debt of defaunated insects.

In the roaches investigated 1 day after defaunation the excess oxygen uptake of recovery is only a little more than the oxygen missed. At 6 days after defaunation the insects repay twice the oxygen debt, while at 8 days the percentage repayment approaches even closer to the value for normal insects. The rate of aerobic respiration remains high in the insects studied at 6 and 8 days after defaunation, as it had been in those at 1 day.

In Fig. 1 the results of all the oxygen debt experiments done at 5° C. are presented graphically, as total oxygen consumed against time. All the results have been equated to the same base-line, and the oxygen consumed per unit time in the recovery period is expressed as a percentage of the initial rate. The graph illustrates the depression of the curve in freshly-defaunated insects and the return towards the normal curve in experiments done at longer intervals after defaunation.

In the course of these experiments a few insects were encountered in which defaunation was not complete. Apparently at the time of

oxygen treatment they had contained cysts,[2] which had not been destroyed. When they were examined at the end of the oxygen debt experiments, the intestine was found to contain numerous cysts, although the number present was much smaller than the normal population of

TABLE IV

Repayment of oxygen debt in defaunated insects.

The duration of anaerobiosis was approximately one hour in each case. Any slight deviations from this time have been taken into account in calculating the oxygen missed.

Experiment	Temp. °C.	Initial O_2 uptake $mm^3./gm./hr.$	O_2 missed $mm^3./gm.$	Final O_2 uptake $mm^3./gm./hr.$	Excess O_2 consumed $mm^3./gm.$	Excess O_2 consumed / O_2 missed × 100	Remarks
B 18 (i)	5.0	34	37	31	50	140	
B 18 (ii)	"	51	55	58	63	110	
B 18 (iii)	"	32	35	32	45	130	1 day after defaunation
B 18 (2)	"	53	57	48	82	140	
B 29 (ii)	"	59	59	66	73	120	
B 29 (iii)	"	36	36	42	57	160	
B 32 (i)	"	40	40	45	54	140	
Average		44	46	46	61	130	
B 29 (i)	5.0	38	38	40	80	210	6 days after defaunation
B 29 (2)	"	39	39	43	77	200	
Average		39	39	42	79	200	
B 32 (ii)	5.0	37	37	38	111	300	8 days after defaunation
B 32 (iii)	"	44	44	40	101	230	
Average		41	41	39	106	260	
B 18 (1)	5.0	44	48	43	166	350	Unsuccessful defaunation; cysts present
B 29 (1)	"	52	52	66	180	350	
B 32 (1)	"	46	46	53	91	200	
Average		47	49	54	146	300	
B 1 (iii)	7.5	54	54	58	41	80	Untreated; just moulted

active protozoa. These roaches behaved, as regards the repayment of oxygen debt, just as did the normal insects, repaying three times the amount of oxygen missed (Table IV).

[2] True cysts are formed in *Trichonympha*, but the corresponding stage in *Barbulanympha* is a rounded resistant form without a cyst wall. Cf. Cleveland, *et al.* (*loc. cit.*), pp. 236 and 262.

One other insect deserves special mention (Table IV, *B* 1 (iii)). It was an untreated roach, but had undergone a moult just prior to the time of the experiment. This individual showed the lowest percentage repayment of debt of all (80 per cent). Although the contents of the gut were not examined, this insect also presumably contained resistant forms, this being the means by which the protozoa are enabled to maintain themselves from one instar to the next.

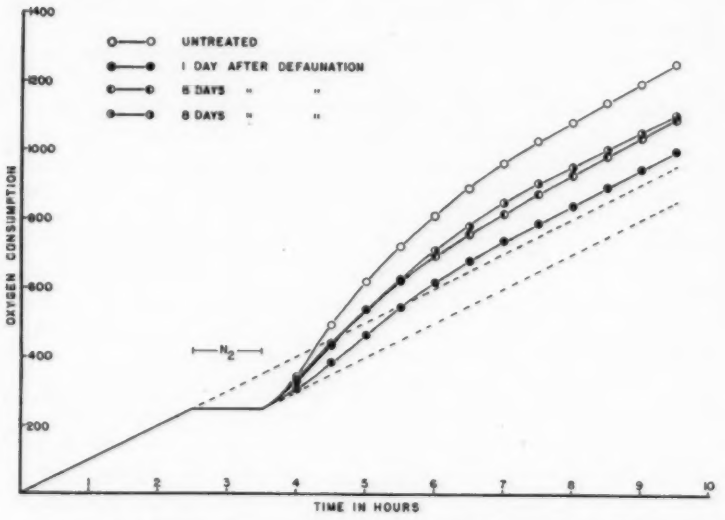

FIG. 1. Repayment of oxygen debt in normal and defaunated *Cryptocercus;* averages of all experiments at 5° C. Ordinates represent total oxygen consumed. The initial oxygen uptake per gram per hour has been made equal to 100 in each group.

Carbon Dioxide Excretion During and After Anaerobiosis.—Experiments on the excretion of carbon dioxide by defaunated insects during 1 hour of anaerobiosis yielded the following results:

(cu. mm. per gram) 84 88 72 52 average 74.

These experiments were done on insects which had been defaunated 7 days previously. The average is not significantly different from the figure for carbon dioxide excretion during anaerobiosis in normal insects. Actually one would expect it to be less than the value for normal insects by an amount equal to the carbon dioxide production of the protozoa. But since the latter would amount to only 5 to 10 cu. mm.

per gram, it would require a large number of experiments to establish such a difference.

A series of determinations of respiratory quotients at successive hours after anaerobiosis was also done on defaunated insects, and the results are presented in Table V. There are fewer experiments than in the series on normal insects and the averages consequently show a less uniform trend. These experiments were carried out either 4, 5 or 7 days subsequent to the defaunation of the insects. The average oxygen uptake of insects investigated 6 days after defaunation was therefore used in determining carbon dioxide excretion, since these figures would approximate most closely to the oxygen uptake of the insects used in the respiratory quotient experiments. The amount of carbon dioxide retained, determined on this basis, is 81 cu. mm. per gram, exactly the same as the carbon dioxide retention of normal insects.

TABLE V

Respiratory quotients at successive one-hour periods after anaerobiosis. Defaunated insects.

Hourly periods	1	2	3	4	5	6
R.Q.	0.23	0.73	0.72	0.59	0.66	0.83
	0.31	0.44	0.26	0.49	0.57	0.99
	0.22	0.64	0.70	0.54	0.66	0.50
	0.19	0.37	0.69	0.39	0.74	0.77
	0.37	0.50	0.81	0.69	—	—
Average	0.26	0.54	0.64	0.54	0.66	0.77

DISCUSSION

It is apparent that the anaerobic gaseous metabolism of *Cryptocercus* differs in almost every particular from that of *Zootermopsis*. In the first place, the carbon dioxide retained in recovery is equal, both in untreated and defaunated insects, to the carbon dioxide excreted during anaerobiosis, which suggests that the latter is derived entirely from the acidification of bicarbonate. It is thus possible that the anaerobic processes in *Cryptocercus* consist entirely of lactic acid glycolysis.

Another point of difference between the two insects is in the percentage repayment of the oxygen debt. Furthermore, there is a decided disagreement in *Cryptocercus* in the amount of repayment between normal and defaunated individuals. A suggestion of minor differences of this nature were obtained from the experiments on *Zootermopsis*, but nothing of the magnitude and regularity of the differences found in

Cryptocercus. It may be stated, broadly, that the removal of *active* protozoa from the gut in some way affects the metabolism so that the percentage repayment of oxygen debt is at first much reduced, although this effect wears off gradually. It is difficult to find an explanation for this phenomenon which would fit all the facts. It seems improbable that defaunation has produced any profound change in the nature of the anaerobic processes, since carbon dioxide excretion during and after anaerobiosis is not altered from the normal.

It is possible that the difference in the repayment of oxygen debt is associated with the increased aerobic respiration of defaunated animals. The oxygen uptake of normal insects is 29 cu. mm. per gram per hour and the carbon dioxide production is 32 cu. mm. per gram per hour, while in defaunated individuals carbon dioxide production is 31 cu. mm. per gram per hour and oxygen uptake is 45 cu. mm. per gram per hour. This suggests that the change following defaunation might have been simply the addition of some process which consumes oxygen without producing carbon dioxide. If this were the case, it would be incorrect to compare the excess oxygen uptake of recovery to an oxygen debt based on the previous aerobic respiration. That there is no change in carbon dioxide production per gram coincident with the removal of the protozoa is not surprising, since there is at the same time a loss of weight representing probably the weight of the protozoa, there being no compensation as there was in the termite, by the addition of an equal weight of non-living material.

The results from insects investigated at 6 and 8 days after defaunation and from those in which cysts were present cannot be explained on these lines, however, since the repayment of the debt was high, in spite of a high rate of oxygen uptake. Perhaps defaunation produced some change in the basal energy requirements in anaerobiosis.

The exaggerated repayment of the oxygen debt in *Cryptocercus* is interesting in the light of the conclusion of Davis and Slater (1928) that in the common cockroach all the lactic acid produced in anaerobiosis is oxidised during recovery, none being resynthesized to glycogen. Davis and Slater found only a fifth of the amount of lactic acid they had expected, and if the rate of aerobic respiration they observed was the actual basal rate, lactic acid glycolysis could have represented only part of the anaerobic processes. Alternatively, if glycolysis were the only mechanism, the aerobic respiration they observed must have been about five times as great as the actual basal value, since an anaerobic mechanism depending entirely on glycolysis, in which all the lactic acid was oxidised during recovery, would entail a repayment of the debt many times over.

The only other attempt to arrive at a quantitative estimate of the repayment of the debt in insects seems to be that of Bodine (1928) on grasshoppers. The duration of anaerobiosis was not very accurately controlled, and the repayment was sometimes less, sometimes more than 100 per cent. Bodine stated that "in carefully-controlled experiments," it could be shown to be approximately equal to the oxygen debt.

If the anaerobic metabolism in *Cryptocercus* is maintained by glycolysis, as the results suggest, the course of the oxygen uptake during recovery means that a large proportion of the lactic acid is being burned. The anaerobic metabolism of *Cryptocercus* thus resembles more nearly that of other roaches than it does that of the termite, which agrees so closely with it in the method of nutrition.

The association of a high carbon dioxide retention with a high percentage repayment of the oxygen debt in *Cryptocercus* and a low carbon dioxide retention (particularly in relation to the anaerobic carbon dioxide excretion) with a low percentage repayment of oxygen debt in *Zootermopsis* suggests that the repayment of an oxygen debt is perhaps, in fact, related specifically to the removal of lactic acid during recovery.

Summary

Fully-grown specimens of *Cryptocercus punctulatus* Scudder had an average oxygen consumption of 46.1 cu. mm. per gram per hour at 7.5° C. and 28.5 cu. mm. per gram per hour at 5° C. The average respiratory quotient was 1.09.

The production of combustible gases by the intestinal protozoa was found to be much less than that previously reported for the termite, *Zootermopsis*.

Complete exclusion of oxygen was fatal to the insects if continued for more than 5 or 6 hours.

The extra oxygen consumed during oxidative recovery from a period of anaerobiosis lasting 1 hour was, on the average, three times as great as the amount of oxygen missed. Recovery was complete at the end of 4 hours after the re-admission of air.

The carbon dioxide excreted during anaerobiosis was 80 cu. mm. per gram in 1 hour at 5° C. and was equal to the amount of carbon dioxide retained during recovery, indicating that the carbon dioxide evolved during anaerobiosis was probably all derived from the acidification of bicarbonate.

In defaunated roaches the average oxygen consumption at 5° C. was 44.6 cu. mm. per gram per hour. The respiratory quotient was 0.69.

In insects investigated 1 day after defaunation the extra oxygen consumed during recovery from anaerobiosis amounted to only 130 per

cent of the oxygen debt. At 6 days after defaunation 200 per cent of the debt was paid off, and at 8 days, 260 per cent.

Insects which had undergone the defaunation treatment, but in which defaunation was not complete, owing to the presence of resistant stages of the protozoa, had a heightened rate of oxygen consumption but behaved like normal insects, paying off 300 per cent of the debt.

The anaerobic carbon dioxide excretion and carbon dioxide retention during recovery of defaunated roaches were the same as those of untreated individuals.

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CELL TYPES AND CLOTTING REACTIONS IN THE ECHINOID, MELLITA QUINQUIESPERFORATA

CAZLYN G. BOOKHOUT AND NELLIE D. GREENBURG

(From Duke University, Ward-Belmont School, and Duke University Marine Laboratory, Beaufort, N. C.)

Introduction

Most of the investigations of the cellular elements and morphology of clotting in Echinoidea have been done on sea-urchins and heart-urchins (Geddes, 1880; Cuénot, 1891; Kindred, 1921, 1924) with an occasional reference to sand-dollars (Kindred, 1924), but none to *Mellita quinquiesperforata*. It is the object of this work to study the cellular elements in the perivisceral fluid of *Mellita* and to determine the sequence of events in clotting under normal and experimental conditions.

The factors which will induce or inhibit clotting in the blood of vertebrates have been the object of numerous investigations, but little work of this nature has been done on invertebrate blood. The work of Donnellon (1938) on *Arbacia* is the only one which has considered experimentally the factors responsible for agglutination of cellular elements in Echinoidea.

Material and Methods

Mellita quinquiesperforata used in this study were obtained by dredging in the coastal waters of Beaufort, N. C. The supply, renewed every other day, was kept in running sea water in laboratory tanks.

To determine the types of cells present, fluid was drained from the perivisceral cavity, a drop placed on a coverslip, inverted over a depression slide, and examined immediately under oil immersion. Camera lucida sketches were made of each cell type. Cells of each type were measured with an ocular micrometer, and their dimensions calculated from twenty of these random measurements.

In order to study phagocytosis, 1 cc. of finely granulated carmine suspension in sea water was injected by use of a hypodermic syringe through the peristomial membrane into three sections of the perivisceral cavity. After definite time intervals, one-half hour to five days, hanging-drop preparations of the perivisceral fluid were examined under oil immersion for phagocytic cells.

This same method was followed in studying fat ingestion. Hard-boiled egg yolk was ground in a mortar, and 1 cc. of a sea water suspension was injected into each animal. Olive oil was emulsified with sea water and similarly injected. Periods of three to twenty-seven hours were allowed for ingestion. One per cent osmic acid and an alcoholic solution of Sudan III were used as fat stains.

One per cent solutions of all vital stains were made up with sea water and allowed to settle. One cubic centimeter of a 1 : 100 dilution of each was injected orally into isolated animals. Hanging-drop preparations were made after periods of three to twenty-seven hours, and examined to determine which cells had stained.

To follow accurately the sequence of events in clumping, hanging-drop preparations were made and examined immediately after fluid was removed from the perivisceral cavity, and observations were continued until all cells were involved in clumps. Although clumping is a continuous process, for descriptive purposes it was possible to recognize four phases designated as stage one, two, three, and four (see p. 312). These four stages were used as criteria in judging the rapidity of the clumping of cells when salts were added to the perivisceral fluid. All salts used were Merck reagents. These were prepared in isotonic solutions. The hypertonic salt solution was prepared by adding 8 cc. of 2.5 M NaCl solution to 50 cc. of sea water.

Two cubic centimeters of perivisceral fluid were drained from an animal into a watchglass containing ½ cc. of salt solution. The clumping process was observed under high power objective of the microscope, and the number of seconds necessary for the mixture to reach each of the four clotting stages as timed by a stop-watch was recorded. At least 15 trials involving 15 animals were made with each salt solution. Controls on normal fluid were run simultaneously by substituting sea water for salt solution.

Observations

Cell Types

There are four types of amoebocytes and two varieties of leucocytes in the perivisceral fluid of *Mellita*. The amoebocytes are constantly changing shape and crawl about by formation of blunt pseudopodia. They move with a fair degree of rapidity.

Amoebocytes filled with red spherules average 20.7 μ in length and 8.6 μ in width, and are the most common type (Fig. 1). Those with colorless spherules are approximately the same size (22.9 μ long and 10 μ wide), and behave similarly (Fig. 2). Amoebocytes with variously

shaped, scattered, brown particles are present in smaller numbers than the first two types mentioned. They average 15.7 μ in length and 8.6 μ in width (Fig. 3). The brown particles in the cytoplasm exhibit continuous Brownian movement. A fourth type of amoebocyte filled with yellowish-brown spherules sometimes appears (Fig. 4). This type is more apt to be found after the sand-dollars have been kept in the laboratory for several days. Spherules similar to these are sometimes found in leucocytes with lobed pseudopodia.

There are two varieties of leucocytes; one has large hyaline ectoplasmic lobes and a small central granular endoplasmic portion (Fig. 5); the second type is spherical and is composed of many vesicular compartments, but lacks visible pseudopodia (Fig. 6). The first variety is 32.1 μ long and 27.9 μ wide; the second, 28.6 μ long and 27.9 μ wide. In the vesicles of the second variety may be seen a small amount of homogeneous pinkish material and occasionally small scattered granules which move about by Brownian movement.

Phagocytosis

One-half hour after injection of carmine into the perivisceral cavity small amounts of carmine particles are present in the amoebocytes with brown particles and in two types of leucocytes. Carmine is still present in the same cells but in much larger quantities after five days (Figs. 7, 8).

The amoebocytes with red and white spherules are similar in size, shape, and behavior, but differ in color and fat content as indicated by their reaction to osmic acid and Sudan III. It was thought that if an emulsion of egg yolk or olive oil were injected into the perivisceral cavity, amoebocytes with white spherules might be the only cells to ingest fat and they would change into amoebocytes with red or colored spherules. This was not the case; the leucocytes (Figs. 9–11) were the only cells to ingest fat.

Reaction to Vital Stains

Three hours after injection of vital stains cellular elements were examined. Safranin, sodium carminate, and methylene blue give negative results. Neutral fuchsin, however, stains the brown movable granules in the leucocytes red and similar granules in the brown amoebocytes pink. Neutral red stains the granules in both types of leucocytes red, but does not stain the granules of the brown amoebocyte. At the end of this period all cells are still viable and appear normal.

Normal Clotting

No fibrin or gelation of the plasma is observed in the normal perivisceral fluid. When the plasma is separated by filtration from the cells it remains as a clear filtrate and undergoes no visible change. Thus clotting in *Mellita* involves the cellular elements alone; all cell types, however, are involved. The first indication of clumping occurs 78 seconds after the fluid is removed from the perivisceral cavity. This phase, designated as stage 1 (Fig. 12), is characterized by clumping of a leucocyte with lobed pseudopodia either with one or two like cells or with cells of the other types. The chance meeting and adherence of other cells enlarge the clump (Fig. 13) in stage 2. In the third stage the clump becomes larger and more compact; few free cells are seen in the field. This stage is reached at an average of 145 seconds after removal from the perivisceral cavity. Occasionally an amoebocyte in which red spherules have broken down may be seen (Fig. 14*a*). Usually, however, amoebocytes with spherules or amoebocytes with brown granules continue to move about after they enter the clump. Amoebocytes with spherules are frequently seen radiating out from the clump attached only by a narrow stalk (Fig. 14*b*). It may be noticed that all the spherules are near the distal portion of this cell, and it looks as if this amoebocyte is attempting to free itself from the clump. Occasionally amoebocytes do free themselves, but they are usually retained by an adhesive substance of the leucocyte with lobed pseudopodia.

EXPLANATION OF PLATE I

All figures were drawn with aid of camera lucida. × 1053.

FIG. 1. Three amoebocytes with red spherules to show differences in size and shape.

FIG. 2. Amoebocyte with colorless spherules.

FIG. 3. Amoebocyte filled with brown fluid containing scattered brown granules.

FIG. 4. Amoebocyte with yellowish-brown spherules.

FIG. 5. Leucocyte with large clear pseudopodial lobes.

FIG. 6. Spherical leucocyte without visible pseudopodial projections. Compartments may possess granules which exhibit Brownian movement as in the amoebocyte shown in Fig. 3.

FIGS. 7 AND 8. Leucocyte with lobed pseudopodia and spherical leucocyte showing carmine grains near the center of the cells. Taken from animals which had been injected 5 days previously.

FIG. 9. Leucocyte with pseudopodial lobes which has engulfed a droplet of olive oil stained with osmic acid.

FIG. 10. Spherical leucocyte with engulfed droplet of olive oil stained black with osmic acid.

FIG. 11. Leucocyte with lobed pseudopodia which has engulfed a bit of emulsified egg yolk stained orange with Sudan III.

FIG. 12. First stage of clotting: two leucocytes with lobed pseudopodia and an amoebocyte with red spherules.

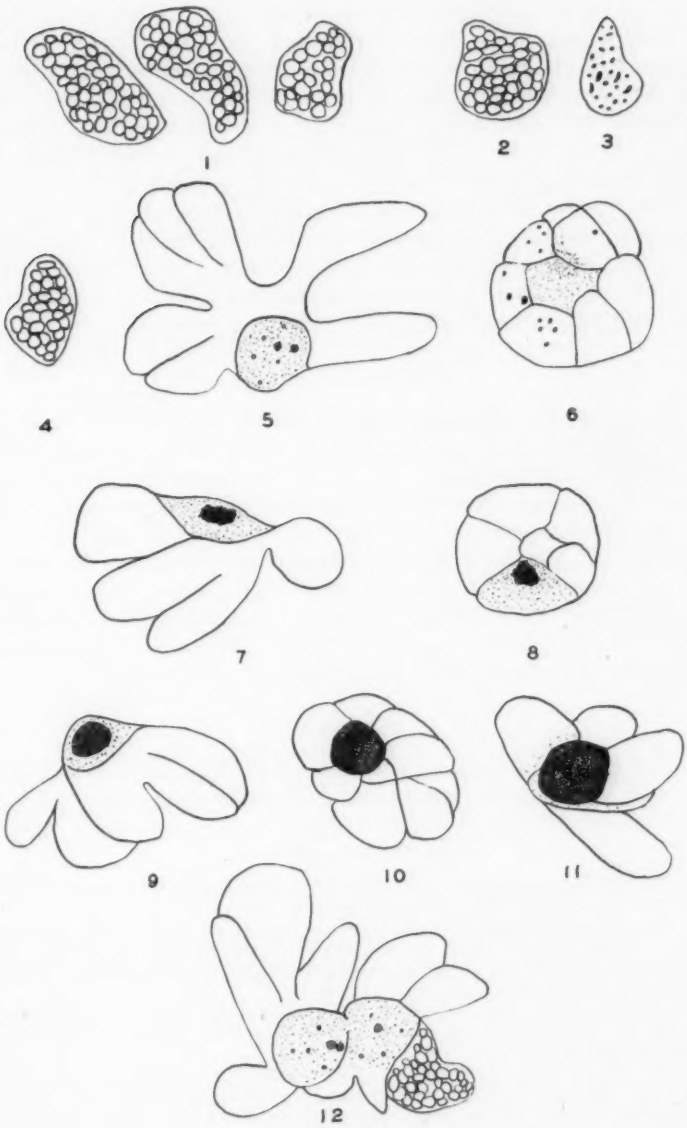

PLATE I

The clumps are large enough to be seen with the naked eye in the fourth stage. They appear as isolated, dark red, opaque masses. Microscopically it can be seen that there is a general breaking down of all cells, especially those near the center of the clump (Fig. 15). The spherules of nearly all the amoebocytes have blended into compact homogeneous masses which often appear trilobed or bilobed before becoming spherical (Figs. 15 *a, b, c*). Only occasionally can an amoebocyte with intact spherules be seen (Fig. 15*d*). The spherical leucocytes are little affected by the general contraction of the whole mass (Fig. 15*e*) but retain their identity. Since no free cells are present, this stage is considered the end-point of clotting and is reached on an average of 165 seconds after removal from the perivisceral cavity.

Action of K and Ca

In an attempt to determine the mechanism which is involved in the clotting reaction of the perivisceral fluid, certain salts were added which might influence the clotting process. The salts used were isotonic solutions of potassium chloride and calcium chloride. These were found to decrease noticeably the time required for clotting (Table I). The results show that the addition of potassium ions decreases the time of clotting much more than does the addition of calcium. When calcium is precipitated by potassium oxalate or rendered slightly ionized by sodium citrate, clotting is greatly inhibited (Table I). Potassium oxalate was more effective than sodium citrate in this respect.

Fat Solvent

A 5 per cent solution of ethyl alcohol decreases clotting time 20.6 per cent. The cells in alcohol solution reached the end-point of clotting

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EXPLANATION OF PLATE II

FIG. 13. Second stage of clotting: larger aggregation of cells including leucocytes and amoebocytes with red and white spherules.

FIG. 14. Third stage of clotting: cellular aggregation larger than in previous stage. *a*, amoebocyte in which red spherules have broken down. Cell is filled with red homogeneous material; *b*, amoebocyte with red spherules appears to be attempting to leave the cellular aggregation.

FIG. 15. Fourth stage of clotting. The cellular aggregation has contracted, most of the amoebocytes have become spherical and their spherules broken down into homogeneous masses.

R, amoebocyte with red spherules broken down; *W*, amoebocyte with white spherules broken down; *Y*, amoebocyte with yellow spherules broken down. *a, b, c*, amoebocytes whose spherules have broken down and which now appear as tri-lobed, bi-lobed, or spherical homogeneous masses; *d*, amoebocyte with colorless spherules still intact; *e*, spherical leucocyte unchanged.

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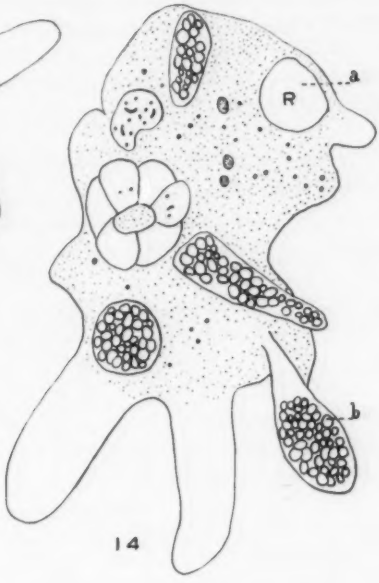

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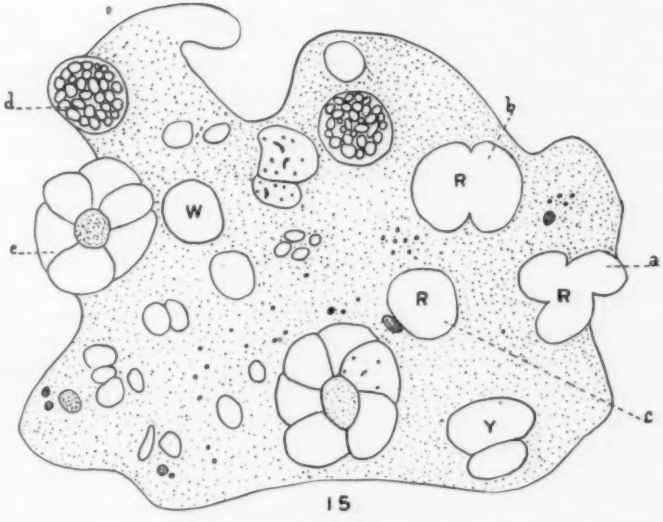

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PLATE II

135 seconds after removal from the perivisceral cavity as compared with 170 seconds in the control.

Hypertonic Salt Solution

A hypertonic salt solution accelerated clotting 45 per cent. The end-point was reached after 95 seconds as compared with 173 seconds in the control.

Action of Magnesium Sulphate

A saturated solution of $MgSO_4$ inhibited clotting indefinitely.

TABLE I

Effect of salts on clotting time of perivisceral fluid of Mellita.

Seconds required for reaction									Percentage Change in Final Clotting Time
Stage of Clotting	First Stage		Second Stage		Third Stage		Fourth Stage		
Salts	with salt	Control	with salt	Control	with salt	Control	with salt	Control	
0.53 M KCl	*		54	90	69	135	77	168	−54
0.3 M $CaCl_2$	66	72	85	89	101	130	117	165	−29
0.27 M Na Citrate	124	69	150	91	181	140	239	171	+40
0.3 M K Oxalate	129	80	151	85	169	142	275	169	+63
Hypertonic NaCl †	63	75	77	80	86	135	95	173	−45

* Too rapid to determine accurately.
† 8 cc. of 2.5 M NaCl to 50 cc. of sea water.

DISCUSSION

Various investigators (Geddes, 1880; Cuénot, 1891; Kindred, 1924) have described amoebocytes with colorless spherules, red spherules, and yellow spherules in sea-urchins. It is interesting to note that in *Mellita* there is a fourth type, a brown fluid-filled amoebocyte with scattered brown granules. This type was not found by Kindred in his comparative study of echinoderms which included the sand-dollar, *Echinarachnius eccentricus*. Geddes (1880), however, described and figured a similar type of cell in the perivisceral fluid of the heart-urchin, *Spatangus purpureus*. Brown amoebocytes as well as two types of leucocytes are phagocytic, as shown by their ability to ingest carmine grains.

They may, therefore, be important in ingestion and transportation of food particles. When the origin of the granules present in the brown amoebocytes as well as similar granules occasionally found in the vesicles of the spherical leucocyte is known, we may be able to determine whether these are food particles which are in the process of preliminary digestion or are excretory granules.

Amoebocytes with red spherules are the most common type in the perivisceral fluid of *Mellita* and Kindred (1924) reports that they are most numerous in *Strongylocentrotus franciscanus,* but less numerous in *Strongylocentrotus droebachiensis* and in *Echinarachnius eccentricus.* In the latter genus (Kindred, 1924) and in *Spatangus purpureus* and *Echinocardium* (Geddes, 1880) amoebocytes with yellow spherules are most abundant. Thus no generalization can be made as to the relative number of each type of amoebocyte in Echinoidea.

There is a theory that red and yellow amoebocytes are carriers of food. Awerinzew (1911) noticed that a variety of *Strongylocentrotus droebachiensis* which lived upon a rock and mud bottom had a greenish-yellow coloring in the skin; another variety which lived among red algae had a red appearance. Injections of ammonia-carmine and India ink revealed that amoebocytes took up these substances and deposited them in the skin. Hence Awerinzew believed that the red color in the skin and in the amoebocytes was the pigment from the red algae. The habitat of *Mellita* is sandy bottoms where there is little if any red algae, and yet the amoebocytes with red spherules are so numerous that they give the perivisceral fluid a reddish tinge. If Awerinzew's conception were correct amoebocytes would take up carmine grains, as he thought; however, this function has been found to be confined to the leucocytes by Kindred (1924) and to the leucocytes and small brown amoebocytes in our work on *Mellita.*

Tests with osmic acid substantiated by Sudan III reveal that amoebocytes with red spherules contain fat, but injection of fatty substances into the perivisceral fluid failed to reveal that it is ingested directly by any amoebocyte.

Cuénot (1891) observed vibrating cells in the perivisceral fluid of all the sea-urchins and heart-urchins he examined. These cells are not present in *Mellita* and Kindred (1924) found none in *Echinarachnius eccentricus.* In this respect the perivisceral fluid of sand-dollars which have been examined differs from that of other Echinoidea.

The presence of two varieties of leucocytes in *Mellita* is not strictly comparable to the condition found by Kindred (1924). The spherical leucocytes (" bladder-amoebocytes " of Théel, 1921) without visible pseudopodia but with vesicular compartments were not found in any

echinoderm examined by Kindred although the leucocytes with large hyaline ectoplasmic lobes present in *Mellita* were found in all classes of Echinodermata he examined. This latter variety normally possesses broad rounded lobes as Goodrich (1919) maintains, and does not normally form long filiform processes as Geddes (1880) and Cuénot (1891) described.

We have considered the spherical leucocyte a different type from the leucocyte with hyaline ectoplasmic lobes although it is possible that they may be different phases of the same cell. They are considered different types not only because of morphological differences but because the leucocyte with lobed pseudopodia is the only one to take part in the formation of a clot.

Clumping of the cellular elements in the perivisceral fluid of *Mellita* is apparently associated with the sticky exterior or adhesive secretion given off by the leucocyte with lobed pseudopodia. Amoebocytes lack this substance because when they make contact with each other they can separate, but if they come in juxtaposition with a leucocyte with lobed pseudopodia they are usually retained even though they attempt to free themselves. Schäfer (1882) described a coagulable material related to mucin which was given off by leucocytes in sea-urchins. He believed this material to be responsible for clumping. Donnellon (1938) thought that the breakdown of the red spherules of the amoebocytes in *Arbacia* liberated something which had an effect upon clotting. This may play some part, but it is very questionable whether it is important in *Mellita* because it does not occur until the end-point is nearly reached. The clot is consummated in *Mellita* on an average of 165 seconds after the perivisceral fluid has been removed from the body. The end-point of clotting in *Arbacia* (Donnellon, 1938) does not occur until 2,760 to 5,300 seconds.

Donnellon (1938) found that certain salts, such as KCl, $CaCl_2$, a hypertonic solution of NaCl, as well as fat solvents decrease the time of clotting, whereas K-oxalate and Na-citrate inhibited clotting in *Arbacia*. Using the same concentrations and molar values, we have been able to confirm Donnellon's findings. The accelerating or retarding effects were in general the same although the lengths of time involved were different. It should be noted, however, that in our work ethyl alcohol was not as effective in accelerating clotting as were the salts. The results indicate that the factors involved in clotting are somewhat similar in the two animals.

When the perivisceral fluid of *Mellita* is first removed there is little indication of clumping which denotes that leucocytes with lobed pseudo-

podia do not have a sticky exterior when in the perivisceral cavity, or if they do it is not strong enough to entrap other types of cells. Why then do these cells become sticky? It is well known that the blastomeres of developing eggs fall apart when grown in calcium-free sea water. The cement which holds the cells together is apparently due in large measure to calcium (Heilbrunn, 1937). Our experimental work indicates that when calcium ions are removed or are slightly ionized clotting is retarded. Thus calcium may be associated with the sticky substance given off by the leucocytes with lobed pseudopodia.

Our experimental work shows that factors other than calcium are important in the clotting process of *Mellita*. Howell (1912) reported that lacerated or wounded tissues emit a substance, "cephalin," which is essential to clotting in vertebrates, and Donnellon (1938) found that tissue extracts hastened clotting in *Arbacia*. In the light of these investigations it seems as though the tissue factor may be important to clotting in *Mellita*. Further experimental work may eventually determine that substances from the tissue factor release calcium from the leucocytes with lobed pseudopodia, render them sticky, and initiate clotting.

We wish to thank Dr. G. W. Wharton for collecting most of the sand-dollars used.

Summary

1. There are two types of leucocytes in the perivisceral fluid of *Mellita*. The first type is characterized by large ectoplasmic pseudopodial lobes and a small granular endoplasmic center. The second type is spherical and is composed of many vesicular compartments, but lacks visible pseudopodia. Both types are phagocytic, but the first type is the only one important in the clotting process.

2. There are three types of amoebocytes filled with spherules: those with red spherules are most abundant, those with colorless spherules are common, and those with yellow-brown spherules are rare. None of these are phagocytic. A fourth type of amoebocyte, smaller than the first three types, is filled with brown fluid and contains small scattered granules which exhibit Brownian movement. This type is phagocytic.

3. Clotting involves the cellular elements of the perivisceral fluid, but does not include the plasma. As soon as the perivisceral fluid is removed from *Mellita* the leucocytes with large pseudopodial flaps produce a sticky substance to which amoebocytes or other leucocytes adhere and finally become entangled into small clumps. As the endpoint is reached there is a general contraction of the clump, entangled amoebocytes become spherical, and their spherules break down. The

end-point of clotting is reached at an average of 165 seconds after the perivisceral fluid is removed from the body.

4. KCl, hypertonic solution of NaCl, $CaCl_2$, and a 5 per cent solution of ethyl alcohol decrease the time of clotting.

5. Na-citrate and K-oxalate increase the time of clotting, and $MgSO_4$ retards clotting indefinitely.

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ANNUAL FLUCTUATIONS IN THE ABUNDANCE OF MARINE ZOÖPLANKTON [1]

MARY SEARS AND GEORGE L. CLARKE

(From the Department of Zoölogy, Wellesley College; the Department of Biology, Harvard University; and the Woods Hole Oceanographic Institution)

From the time of the earliest oceanographic expeditions, it has been recognized that the open oceans are more densely populated in high northern and southern latitudes than in the tropical belts, and that the richest areas of all are boreal coastal waters. But even in these fertile zones, the abundance and character of the plankton is known to fluctuate from time to time. Perhaps the most obvious change is a seasonal one. During the spring a wave of reproduction commonly follows the winter period of scarcity, and later in the summer there may or may not be a second period of increase. However, the seasonal cycle is not constant from year to year either in the numbers or in the species of animals produced. In certain cases, annual changes of this sort have been thought due to fluctuations in the amount of " foreign " water entering a given area from neighboring regions. As a consequence, plankton studies in recent years have frequently centered on a search for " indicator " species which would reveal the presence of intruded water and give a clue to its origin. Possibilities were foreseen of the practical use of such " indicators " in forecasting the success of the fisheries or even in solving complex hydrographic problems (for a recent bibliography, see Russell, 1939; additional references, Pierce and Orton, 1939; Redfield, 1939; Sømme, 1934; Bigelow and Sears, 1937; Report Newfoundland Fisheries Research Commission, 1932; Frost, Lindsay, and Thompson, 1933; Thompson and Frost, 1935, 1936).

By far the most extensive plankton investigations based on the study of " indicators " of this sort has been undertaken at Plymouth, England (Russell, 1939). Of the results obtained perhaps the most suggestive are derived from the fluctuations in abundance of the two chaetognaths, *Sagitta setosa* and *Sagitta elegans,* since the former has almost entirely replaced the latter in the years following 1931. At the same time, in the Plymouth region, there has been a continued impoverishment of the phosphates in the water, a scarcity of fish larvae, and finally a failure of the herring fishery. Since it has been concluded that " *S. setosa* pre-

[1] Contribution No. 249 of the Woods Hole Oceanographic Institution.

dominates off Plymouth when the easterly element in the Dover Straits current is suppressed and the flow of Atlantic water into the North Sea from the north is strong (Carruthers theory) " (Russell, 1935, p. 328), the unusual conditions in the plankton are believed due primarily to changes in water circulation.

There are, however, examples of fluctuations among the planktonic animals of the Plymouth region which are not so clearly correlated with a mixing of water masses. Thus, the two species of the siphonophore genus *Muggiaea*—inhabitants of warm coastal waters generally—do not appear together in the waters off Plymouth: *M. atlantica* has been taken regularly at the Seven Stones lightship between 1913 and 1925, whereas *M. kochii* replaced it after 1925—at least until 1934 (Russell, 1934). It is suspected that both these species are " indicators " of coastal waters from the south, i.e., the shallower waters bordering on the Bay of Biscay. If this is true, there must be some factor (or factors) other than transport by currents responsible for fluctuations of species such as these which are presumably inhabitants of the same water mass. Such differences would be extremely difficult to distinguish in an area such as Plymouth, which is beset by strong tidal currents as well as by ocean currents.

It is the purpose of this paper to call attention to the occurrence of marked fluctuations in the zoöplankton on the continental shelf south of Cape Cod, a region which is not subject to such irregular mass incursions of " foreign " water as invade the Plymouth area. Such fluctuations as those just cited for the two species of *Muggiaea* may be due in part, at least, to limiting factors as subtle as those controlling the size of the different year classes among fish.

In the coastal waters from Cape Cod to Chesapeake Bay, plankton samples were collected by the U. S. Bureau of Fisheries on 23 cruises during the first half of the years 1929–1932 (Bigelow and Sears, 1939). These observations have been supplemented by stations on a section crossing the shelf off Montauk Point on ten occasions between October, 1937 and June, 1939 (Clarke, 1940) and by collections made at the whistle buoy 3 miles off Martha's Vineyard in 1935–1936 (Sta. 3, Clarke and Zinn, 1937) and on other occasions during the summers of 1937, 1938, and 1939 (unpublished data). These data clearly show important fluctuations not only in the total mass of zoöplankton, but also in the abundance of some of the more common species. Here, the waters undergo essentially the same seasonal changes in temperature and salinity each year (Bigelow, 1933; Bigelow and Sears, 1935; Clarke, 1940) following the cycle which is characteristic of boreal waters generally. A considerable amount of highly saline water from beyond the

edge of the continental shelf must enter this region each year to compensate for the large inflow of river water and rain (Iselin, 1939). However, any transport of plankton into this coastal area by this "slope" water (Iselin, 1936, p. 11, Fig. 2) is slight, since there is always a sharp boundary between the populations of the coastal waters and those offshore. In fact, on the routine cruises now being run SSE from Montauk Point to a point the other side of the Gulf Stream, it is usually possible to sort the catches by casual inspection, according to the locality of capture. The catches on the continental shelf consist essentially of the common coastal species (see Bigelow and Sears, 1939); while those in the "slope" water are composed of *Metridia lucens*, rather than *Calanus* or *Centropages*, as the outstanding copepod, *Sagitta enflata*, as the characteristic chaetognath, and usually salps (*Salpa fusiformis*, *Iasis zonaria*) in some quantity as well as other typically oceanic species (unpublished data; Clarke, 1940). While *Metridia*, it is true, occurs in some numbers over the outer half of the shelf, particularly in the north, we seldom find other oceanic species more than 10–15 miles inside the 200-meter contour.[2] Hence, while there is some possibility of "contamination" by offshore animals in the region under consideration, it seldom affects any considerable area and never for any length of time.

In addition to the increments derived from the "slope" water, our coastal area also receives water in small quantities from the vicinity of Nantucket Shoals, particularly during the spring. We have little reason to suppose, however, that this has any marked effect on the zoöplankton, for during the spring of 1932, when several intrusions of colder water from the north were observed (Bigelow, 1933), the only noticeable effect of this water movement was the introduction of a meager number of *Calanus hyperboreus* and *Oikopleura labradoriensis* into the area south of Cape Cod (Bigelow and Sears, 1939, p. 247). Actually, during the spring, when flooding from the north most often occurs, the chief effect would be a dilution, since at this season the population in the waters of the Gulf of Maine is at its minimum (Bigelow, 1926). Were there to be an intrusion from this source later in the summer, it is unlikely that the nature of the plankton would be appreciably affected, since catches made in September (1939) on the western part of George's Bank and over the continental shelf between Montauk Point and Martha's Vineyard (unpublished data) were of much the same richness and type, chiefly *Centropages typicus*.

[2] The waters may become "overridden" temporarily with warm-water species which reproduce rapidly (i.e., *Salpa fusiformis*—July 6 to mid-August, 1929). But the enormous quantities of these (3,000–5,000 cc. per 20-minute tow with a meter net) are due to local propagation and are not an indication of an unusually large mixing of "slope" water, because other oceanic species occur only as traces.

Nevertheless in this region between Cape Cod and Chesapeake Bay—an area biologically isolated from the "slope" water offshore and only slightly contaminated by the plankton of Nantucket Shoals—*Calanus finmarchicus* showed a 10-fold fluctuation in abundance (by volume) at the season of its greatest richness during the years 1929–1932 (Fig. 1). In addition, when the richest of these periods (July, 1931) is com-

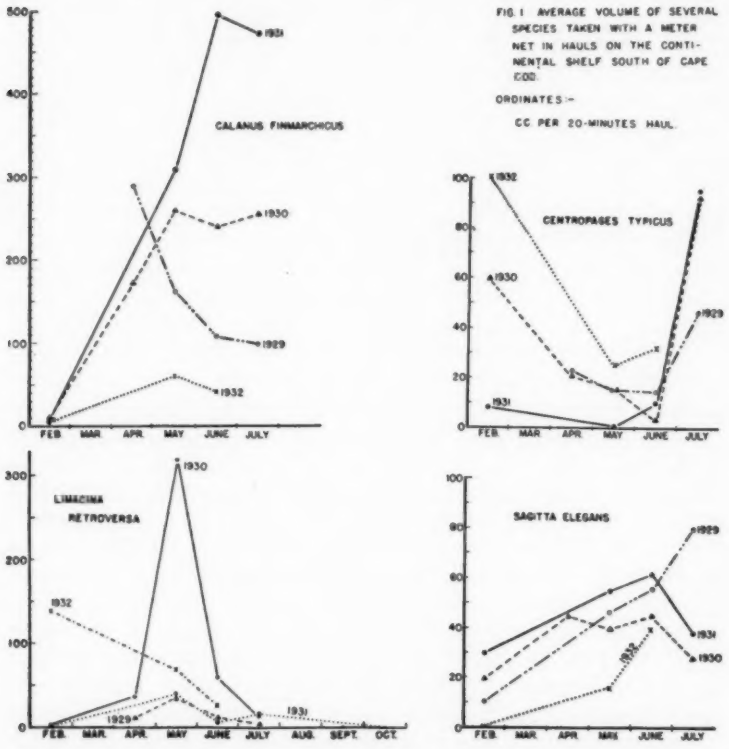

FIG. 1 AVERAGE VOLUME OF SEVERAL SPECIES TAKEN WITH A METER NET IN HAULS ON THE CONTINENTAL SHELF SOUTH OF CAPE COD.
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pared with the very poor periods of a few years later (July 1937–1939), a 600-fold difference (by volume) is revealed. Equally great fluctuations in the stock of *Calanus* have been encountered south of Woods Hole in other years when no extensive surveys were undertaken. This copepod was virtually unobtainable in the vicinity of Woods Hole during the past three summers, although it was abundant during 1935 and 1936 (Clarke and Zinn, 1937).

TABLE I

Species	Year of greatest abundance by volume	Year of least abundance by volume
Calanus finmarchicus	1931	1932
Limacina retroversa	1930	1931
Sagitta elegans	1929, 1931	1932
Centropages typicus	1932	1931
Aglantha digitale	1932	1931
Euthemisto compressa	1930	?

Other individual species studied quantitatively during 1929–1932 have similarly undergone marked fluctuations (more especially over the northern half of the area), notably *Limacina retroversa* (12-fold, Fig.

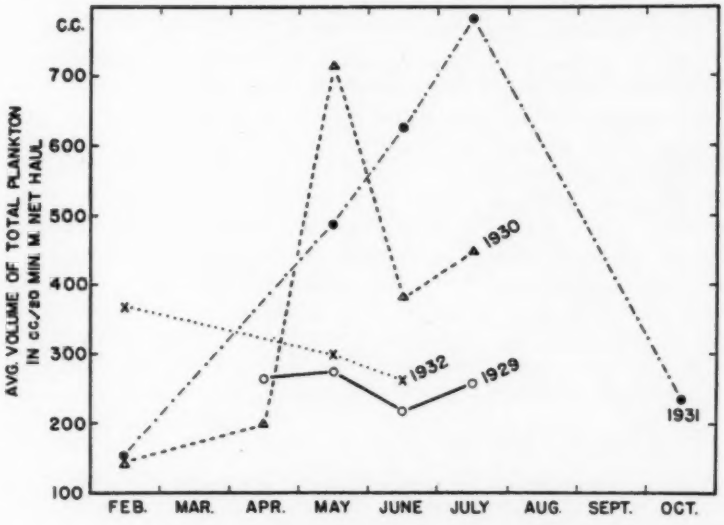

FIG. 2. Average volumes of total plankton.

1), and to a lesser degree *Sagitta elegans* and *Centropages typicus* (Fig. 1). Other members of the plankton which ordinarily occur as odd specimens may become sufficiently numerous in certain years to form a measurable proportion of the total. Such was the case for *Euthemisto compressa* in 1930 and *Aglantha digitale* in 1932. Furthermore, it ap-

pears that these fluctuations occur independently of one another—a year (1931) rich in *Calanus* being poor in *Limacina* or a year (1932) rich in *Centropages* being poor in *Calanus,* etc. (See Table I.)

Not only do individual species exhibit these marked fluctuations, but in addition the plankton as a whole varies in abundance from year to

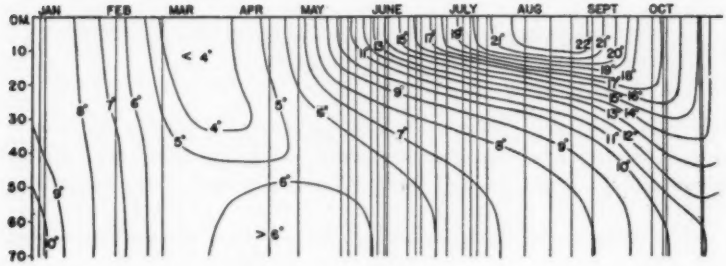

FIG. 3. Average temperatures throughout the year on the continental shelf off Montauk Point, based on all available data.

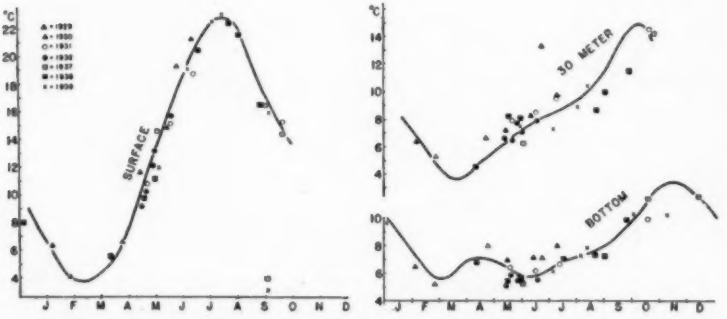

FIG. 4. Departures of temperature from the average seasonal cycle at the surface, 30 meters, and the bottom, for 1929–1932 and 1937–1939, at stations halfway across the continental shelf off Montauk Point.

year (Fig. 2). In the relatively short series of observations (1929–1932), plankton was much more plentiful during the warm winter of 1932 than in the other winters, especially in the south (Bigelow and Sears, 1939), and this condition seemingly continued through late April (judging from a few vertical hauls of about 400 cc. per 20-minute tow—unpublished data). By May and June of that same year, however, the

plankton was distinctly sparse—in fact, as sparse as in 1929. On the other hand, although the plankton had been very poor in the winter of 1931, it was decidedly richer than average during every other month of that year when surveys were made. Earlier surveys had likewise shown similar fluctuations, the plankton being unusually abundant during the cold summer of 1916 and scarce during the warm summer of 1913 (Bigelow, 1926).

Without a longer series of observations we can offer little explanation for these fluctuations. It has been suggested that temperature is the factor in our area which prevents *Calanus* from entering the upper water layers (15–22° C.) in the summer months and restricts this species to the deeper water (7–12° C., Fig. 3). Nevertheless, it does not appear likely that temperature *per se* causes these annual changes in abundance of *Calanus* since the temperature differences are only 1–2° C. between "warm" and "cold" years (Fig. 4)—a difference which would hardly limit the distribution of an animal living over such a wide temperature range as *Calanus*.

We have also made a cursory examination of the salinities and found them to be somewhat more variable locally than the temperatures, from year to year, and even from month to month, especially in the surface layers. However, there appears to be no abnormal mixture of offshore water, the variability seemingly being caused by irregularities in the local rainfall and the inflow from the rivers.

Since the occurrence of wide fluctuations in the abundance of zooplankton in our coastal area now seems well established, further investigation of the consequences of such variation is called for. It is to be hoped, for instance, that a correlation may be found between the richness of the plankton and the fluctuations in the abundance of a commercially important fish such as the mackerel (Sette, 1940), which feeds upon the plankton.

Summary

The abundance of the zoöplankton in the waters overlying the continental shelf between Cape Cod and Chesapeake Bay is shown to be subject to severe annual fluctuations in respect both to individual species and to the total population. Yearly variations of this sort in British waters have been attributed to mass incursions of water from outside sources; but since no great annual differences in the exchange of water masses have been found in the coastal regions treated in this study, our data strongly indicate that important fluctuations in the plankton may occur in relatively undisturbed areas. These changes in the plankton

are not found to be correlated directly with gross environmental changes and therefore must be due to an indirect action of physical or biological factors.

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HETEROPLASTIC TRANSPLANTATION AND SPECIES SPECIFICITY

I. A Comparison of the Effects of Reciprocal Chorio-allantoic Transplants of Macerated and Unmacerated Duck and Chick Kidney Tissue

CARL J. SANDSTROM

(*From the Department of Biology, University College, New York University*)

Heteroplastic transplantations, especially those designed to study the development of species specificity in the embryo, have often led to results of doubtful significance. Frequent contradictions appeared, and interpretations resulted in ambiguities. As a consequence, any correlation between the transplantability of a tissue and species specificity has been questioned. To study this, and contingent problems, a series of transplantation experiments has been planned using hosts and donors of a stage in development when the relation in question first becomes apparent, and presumably, therefore, when it can be most easily analyzed.

In investigating some of the various aspects of heteroplastic transplantations, Sandstrom and Kauer (1933*a*) studied the host reactions incited by macerated duck kidney tissue implanted on the chorio-allantoic membrane of the chick. They discovered that macerated tissue from the early embryo, e.g., 13 days of incubation, could grow and differentiate, and to a certain degree reconstitute itself into what appeared to be an integrated kidney tissue, unhampered by, and without effect on, the host. But as the donor embryo approached the hatching stage, not only did the macerated kidney tissue fail to grow, but there was evidence that it had a lethal effect on the host. While this drastic effect might have developed gradually, it expressed itself rather suddenly at hatching. Accordingly, implantations of macerated kidney tissue from just-hatched ducklings caused a mortality rate of 100 per cent. They attributed the death of the hosts to an anaphylactoid shock brought about by certain species specific qualities which the protoplasm of the donor tissue apparently acquired just previous to the time of hatching. The results were so striking that a more complete investigation, with an attempt to relate the findings to the results of experiments involving intact tissue transplants, seemed desirable.

A small portion of the results was reported in abstract by Sandstrom, Eisen, and Siffert (1939).

Experimental Procedure

Two series of heteroplastic transplantations were made, namely, duck to chick, and chick to duck. In addition, homoplastic transplants were made to both the chick and the duck. Single-combed White Leghorn chickens and White Pekin ducks furnished both donors and hosts. The ages of the chick donors were selected as follows: embryos of 16 and 20 days' incubation, 5-day old chicks, and adults of uncertain age, while those of the duck donors were embryos of 13, 21, 24, and 27 days' incubation, and ducklings, 1, 4, and 7 days old. The chick hosts were embryos of 9 days' incubation, and the duck hosts, of 13 days' incubation.

In all of the transplantations, tissue from the metanephric kidneys was used. The organ was dissected free from adjacent tissues, and kept in normal saline solution at 38° to 39° C. until a sufficient number of metanephroi of any one stage had been obtained. The time required to collect them varied according to the age of the donor, but usually, at most, it was a matter of only a few minutes. The metanephroi were then completely macerated in a small quantity of saline solution by grinding with a pestle in a glass mortar. After this vigorous treatment the contents of the mortar were washed into a centrifuge tube with saline solution, and the ground tissue centrifuged out. By the technique characteristic of chorio-allantoic grafting, a bit of the collected tissue, only 1 cubic millimeter, was implanted on the chorio-allantoic membrane of the host which was then returned to the incubator for continued incubation.

Examinations of the hosts were made at set intervals in various ways. Usually they were examined at some interval within 48 hours after the implantation, either by candling, noting the condition of the inner shell membrane through an opening into the air chamber, or opening the egg and sacrificing the embryo if it were still alive. In some cases the embryos were to be utilized for another type of experiment, and only a few eggs were examined at any one time, so that the observations extended over several days. Whenever such variations in the procedure might have some bearing on the interpretations of the results, they will be mentioned specifically.

In comparing the results obtained after the implantation of macerated, with those of intact tissue, data from other experiments were utilized for the latter. For this reason the ages of the donors of the intact tissue do not always agree with those of the macerated tissue. The deviation, however, is not so great as to offer any serious objections.

Perhaps it is well to point out here that the mortality rate of the developing chick hosts, which forms the basis of this report, might readily be affected by many factors, some of which are undoubtedly the result of operative procedure, although there is reason to believe that the latter is not of considerable consequence. There are in addition some factors which, under ordinary circumstances, are beyond the control of the experimenter. Reference is being made, of course, to the hardiness of the poultry stock, the handling of the eggs, weather conditions in transit, etc. While a check had been made with respect to the source of the eggs, yet there have been instances when comparable ex-

TABLE I

Mortality rate following implantation of macerated kidney tissue

Host-donor relationship	Number of cases	Age of donor	Age of host	Length of time on host	Number dead	Mortality rate
		(in days)	*(in days)*	*(in days)*		*per cent*
Duck-on-chick	14	13	9	2	3	21.4
	56	21	9	2	21	37.5
	28	24	9	1.25	7	25.0
	27	27	9	2	16	59.2
	57	28	9	1.5	50	87.7
	19	32	9	2	19	100.0
	103	35	9	2	103	100.0
Chick-on-duck	31	16	13	2 to 9	14	45.1
	27	20	13	2	14	51.8
	63	26	13	2	33	52.3
	29	Adult	13	2	8	27.5
Duck-on-duck	39	35	13	2	11	28.2
Chick-on-chick	38	26	9	2	9	23.7

perimental treatment was afforded two separate shipments of eggs, but with quite contrasting results. Although no results were considered when there was reason to suspect unfavorable treatment of the eggs that might affect the viability of the embryos before incubation, e.g., sudden extreme changes in temperature while the eggs were in transit from the hatchery to the laboratory, nevertheless, the percentages which express the mortality rate will show, particularly when the numbers are low, considerable variation which must pass unexplained.

RESULTS

The results of the heteroplastic transplantations are summarized in Table I. Perusal of the table reveals that the macerated duck tissue

had little effect on the chick hosts until the age of the donor material approached that of the hatching period, i.e., the twenty-seventh day of incubation. At this time, the day before hatching, the mortality rate among the hosts, resulting from the implantation of the macerated duck tissue, increased to 59.2 per cent. When the macerated tissue was obtained from donors on the day of hatching, there was an increase in the death rate following implantation to 87.7 per cent, and from older stages of donors it was consistently 100.0 per cent. All the deaths occurred within 48 hours from the time of the implantation. It is an escapable fact that some of the embryos died from causes unrelated to the experiment, but the marked effect on the hosts produced by tissue obtained from donors of hatching age, or older, was most decidedly demonstrated.

In the hosts which survived long enough to permit incorporation, a gross and microscopical study of the chorio-allantoic membrane bearing the implanted tissue indicated some rather definite changes from the normal. Most pronounced was the packing, and apparent agglutination, of the erythrocytes in the blood vessels, both arteries and veins. These vessels in some instances were distended as though by an internal pressure. Diffuse blood clots not infrequently marked the area of implantation, and hemorrhages were also found in the embryo itself. The hemorrhages were for the most part subcutaneous, but some were localized in the hind brain.

While the heteroplastic transplantations of macerated duck kidney tissue displayed certain well-defined effects on the chick hosts, the reciprocal relations did not. As indicated in Table I, the macerated metanephric tissue from the chick, regardless of its age, when implanted on the chorio-allantoic membrane of the duck, had no significant effect on the hosts. To be sure, the death rate for the 16-day donor was not actually as great as indicated, for, as given, the percentage includes all of the deaths that occurred through a period extending from 48 hours to 9 days. While it is possible that the mortality rate at the end of the second day could have been equal to that given in the table, this was not true because the degree of development of some of the host embryos was greater than that of 11 days. The significance, however, of what thus might have been interpreted as an increase in the mortality, is immediately dissipated by the pronounced decrease following the implantation of tissue from the adult chicken. Further evidence that no reaction comparable to that resulting from the implantation of macerated duck tissue occurred, was corroborated by a histological examination of the host membrane. It showed no packing of the blood vessels, nor were diffuse blood clots present in the membrane to the extent that they

were in the other group of heteroplastic implantations. Likewise, brain and subcutaneous hemorrhages were lacking.

The two series of homoplastic transplants involving hatched chicks and ducklings indicated no effects on the hosts. The results, as summarized in Table I, compare favorably with those of the chick-on-duck implantations, and duck-on-chick before the donor had approached the hatching stage.

TABLE II

Mortality rate following implantation of intact kidney tissue

Host-donor relationship	Number of cases	Age of donor	Age of host	Length of time on host	Number dead	Mortality rate
		(in days)	*(in days)*	*(in days)*		*per cent*
Duck-on-chick	84	9	9	2 to 9	32	38.1
	94	13	9	2 to 9	46	48.9
	22	19	9	2 to 9	12	54.5
	19	24	9	2 to 9	13	68.4
	43	27	9	2 to 9	16	37.2
	39	Adult	9	2 to 9	4	10.3
Chick-on-duck	41	9	14	2 to 11	6	14.6
	24	13	14	2 to 10	3	12.5
	42	19	14	2 to 10	3	7.1
	16	24	14	2 to 10	9	56.2
	28	27	14	2 to 10	3	10.7
	39	Adult	14	2 to 9	13	33.3
Duck-on-duck	76	13	13 to 14	2 to 10	30	39.4
	36	21	13	2 to 10	10	27.7
	17	24	13	2 to 10	7	41.1
	14	27	13	2 to 10	7	50.0
	27	32	14	2 to 10	9	33.3
Chick-on-chick	62	9	9	2 to 9	18	29.0
	41	19	9	2 to 9	16	39.0
	55	24	9	2 to 9	24	43.6

The effects of the heteroplastic transplantations of the intact, unmacerated kidney tissue are summarized in Table II. The data were compiled from many experiments performed over a period of years, but the general experimental conditions were precisely the same as for the implantations of macerated tissue. The mortality rate, as expressed in this table, however, is not that present after 48 hours, but the total after various periods extending from 48 hours to 9 days. Since many of the hosts showed development beyond the 11-day stage, the percentage that might have expressed the mortality rate at the end of 48 hours can always be assumed to be somewhat less than that recorded. Examina-

tion of the mortality rate shows no significant differences between the various age levels. The same is true for the reciprocal series of chick-on-duck implants. Both sets in the heteroplastic series compare favorably with those in the homoplastic.

Discussion

The results of the implantation of macerated duck tissue to the chick membrane are significant for an understanding of many problems involved in the development of species specificity in the embryo. They emphasize the virtually complete independence of those factors governing the transplantability of a tissue and certain intracellular factors which may be responsible for species specificity, and which become effective only when cell boundaries are broken down. This fact becomes more apparent by a comparison of the effects that macerated duck kidney tissue had on the chick hosts, as included in Table I, with the effects of comparable intact tissue, as summarized in Table II. In the latter group there was no increase in the percentage of deaths following the implantation of tissue from donors of the hatching age, or older. The intracellular factors which were responsible for the effects produced with macerated tissue, although present, were confined within the cell boundaries, and could not, therefore, demonstrate their presence. Furthermore, a comparison of the duck-on-chick transplants of macerated tissue with homoplastic transplants from comparable donors, suggests that the effects on the hosts may be due to factors of importance in species specificity.

Pursuant to the above, it is clear that the problems related to the development of species specificity cannot be adequately analyzed by the utilization of intact tissue transplants only. There is no question that the reaction incited in the tissues of the host by the presence of a bit of engrafted tissue is a response to metabolic substances of a toxic nature, as postulated by Loeb (1930). In other words, it is a typical, localized inflammation reaction. While it is conceivable that a relation exists between the kind and intensity of such reactions and the genetic relationship between the donor and recipient, as contended by Loeb (*loc. cit.*), and further substantiated by Loeb and King (1935), modifying factors are so numerous that they tend to obscure, or confuse the real expression of these relations. For example, Sandstrom (1932) obtained necrosis of intact metanephric tissue of the duck in chorioallantoic grafts on the chick. The necrosis was preceded and accompanied by a typical cellular reaction. On the other hand, Sandstrom and Kauer (1933*b*) found that cartilage from donors of the same species and of comparable, or older stages, displayed no necrosis, and little

cellular reaction from the chick membrane. Although the species specificity factor was unchanged in the two series of transplants, and therefore should have called forth similar reactions, such was not the case. The nature and greater intensity of the reaction against the actively functional metanephric implant was presumably due to a greater production of toxic substances which produce a proportionately greater reaction in the surrounding host tissue. This response interfered with complete incorporation and vascularization, and ultimately led to an absorption of the transplant and its replacement by connective tissue. In contrast, the passively functional cartilage produced relatively little toxin, and the incorporation of the avascular tissue, rapidly accomplished by the mere contact with the host connective tissue, was unhampered by any reaction which might be antagonistic to a foreign tissue.

That the local reaction can be modified in other ways was also demonstrated by Sandstrom (1934), who obtained functional duck kidney tissue equivalent to that of a 2-day duckling from the chorio-allantoic membrane of the chick after it had grown thereon for a period of 11 days. This condition was possible only because of ideal circumstances wherein the implant was properly incorporated and vascularized before local reactions could interfere. As a result, the toxins of the transplant were removed by the functional graft itself. There was, under these circumstances, no evidence of a host reaction. The most ideal conditions for the establishment of a functional transplant which might grow unhampered by the host would be the concomitant growth and differentiation of the transplant and host. Such an ideal was most successfully achieved by Milford (in press), who obtained normal functional duck kidney tissue equivalent in age to 35 days' incubation after having grown as an intracoelomic graft in the chick for a period of 18 days. The storage of excretory products in these instances offers no difficulty because of the characteristic nature of the excretory function in the bird where uric acid is deposited in the cavity of the allantois after the reabsorption of water by the allantoic membrane. The amount of concentrated uric acid produced by a small graft of kidney tissue after the removal of the water would be relatively small, and readily stored in tissue spaces within the host membrane. As pictured by Sandstrom (*loc. cit.*), it is possible for large kidney tissue grafts to be connected directly by means of their tubules to large vesicular-like spaces which facilitates waste removal. Inasmuch as these and many other factors, will modify the local inflammation reaction, considerable care must be exercised in using transplantations for the study of species specificity, and the resulting conclusions, therefore, must of necessity, be very general.

The violent reaction in the chick host caused by the macerated duck tissue was not a localized manifestation, but was distributed widely in the embryonic system through the blood vascular system. The microscopic and gross examinations of the hosts and their membranes revealed pronounced effects on the blood vascular system not only in the area of the implant, but also in the embryo itself. It could not, of course, be ascertained whether these visible effects were primary or secondary, but the general indications were that the implanted material contained substances which caused an agglutination of the blood cells, and led ultimately to the collapse of the respiratory system. Death was therefore presumably the result of suffocation. It is impossible to state whether this phenomenon of agglutination is in any way related to an immunological reaction, perhaps, in some manner, to the development of antigenic properties and antibody production in the developing embryonic donor and host respectively. Only preliminary attempts have been made to determine quantitatively the minimum amount of macerated tissue necessary to produce death. The effective quantity must be very small, because, of the 1 cu. mm. of macerated tissue implanted, only a small part comes in immediate contact with the membrane. Furthermore, in a few preliminary experiments, it has been found that as little as .3 cc. (5 drops) of the unfiltered, supernatant fluid left after centrifuging macerated kidney tissue from a duckling 4 days old, was sufficient to kill the host in less than 30 hours, but none died before 8 hours. In the transplantation of intact kidney tissue pieces of the metanephros were implanted because the entire organ was too large for proper incorporation. It follows that some of the cells must have been injured by cutting, and the question arises whether the release of the intracellular substance, since the effective dose is so small, did not in some manner bear on the transplantability of the intact tissue. Since care was taken to cause a minimum amount of injury, the amount of intracellular substance freed apparently was insufficient to have demonstrable results, although a comparison of the duck-on-chick with the chick-on-duck transplantations of intact tissue (see Table II) reveals in general a lower mortality rate for the latter group than the former, thus simulating the effects of macerated implants. The significance of this difference is questionable, particularly since the percentages are higher in all age groups, and kidney tissue from duck embryos of 27 days' incubation, and even adults, caused no greater percentage of deaths than did the implants from younger duck donors.

While the results of the implantation of the macerated duck kidney tissue to the chorio-allantoic membrane of the chick have been the source of many interesting speculations and additional problems, the implants

of the macerated chick tissue to the membrane of the duck had no effect on the hosts. Such a complete lack of reciprocity is not without precedence, and in this instance was not wholly unexpected. Sandstrom (1936) demonstrated a decided difference between the results of reciprocal chorio-allantoic transplantations of intact metanephric tissue of the duck and chick. A comparison made between the two types of reciprocal implants, i.e., intact and macerated, tends to confound rather than clarify the problems involved. Whereas the macerated duck tissue at certain ages had profound effects on the chick host, the intact tissue of comparable ages, and even from adults, was rapidly incorporated. In the reciprocal host-donor relationship, in which the macerated tissue had no effect, the intact tissue was only slowly incorporated. No explanation is attempted for these seemingly paradoxical results, but they do give emphasis to the contention that transplantability is independent of intracellular properties.

A comparison of the results of the transplantation of intact and macerated tissue of the duck to the embryonic chick host also displays a striking parallelism to the results of experiments which have attempted to correlate transplantability with serological blood properties of the host. Several workers have compared the success of skin grafts to serological relationships. Baldwin (1920) and Kubanyi (1924) support the conclusions of Masson (1918), who seems to have demonstrated that skin implants could be successful only if the donor was of the same blood group as the host. Others, particularly Kozelka (1933), found evidence to the contrary. He made detailed investigations of the problem, transplanting such integumentary structures of the chicken as the wattle, comb, and spur. He found no relation between the success of the transplant and the agglutinating phenomenon of the host's blood. In the assumed parallelism, the integumentary grafts of Kozelka can be compared to the intact kidney tissue. Any apparently antagonistic reaction would be local, and a response of the host's tissue to the metabolic toxins given off by the transplant. It would therefore be independent, or nearly so, of the specificity of either the host or donor. The agglutinating factors of the blood are at least analogous to the intracellular factors released by crushing the metanephric tissue. Both have lethal effects on the host, but are potent only when, and if, they gain entrance into the blood stream.

Summary

1. Metanephric tissue from duck and chick embryos of comparable ages, ground in a mortar and collected by centrifuging, was implanted on the chorio-allantoic membrane of the chick and duck respectively.

2. The implantation of the macerated duck tissue had no significant effects on the chick hosts until the donor embryos approached the age of hatching, at which time it caused death within 48 hours. The average mortality rate following the implantation of tissue from donors of 24 days' incubation, or younger, was 27.9 per cent. When the tissue was obtained from donors of 27 days' incubation, the percentage of deaths among the hosts increased to 59.2 per cent, and when obtained from donors of 28 days' incubation (time of hatching), it increased to 87.7 per cent. Macerated tissue from donors older than 28 days' incubation consistently killed the hosts, the mortality rate being 100.0 per cent.

3. The reciprocal relation, i.e., chick-on-duck, showed no significant increases in mortality rate comparable to that demonstrated in the duck-on-chick implantations.

4. Death of the chick hosts following the implantation of macerated duck tissue resulted from an apparent agglutination of the blood cells by intracellular substances which were released from the cells by maceration.

5. Comparing the effects of the implantation of macerated tissue with those of the intact, it was concluded that the transplantability of a tissue, as manifested by the nature and intensity of the local inflammation reaction incited by metabolic toxins given off by the transplants of intact kidney tissue, is independent of intracellular substances which may be responsible for species specificity, and which are released from the cell only by crushing.

6. Attention was called to the fact that the local inflammation reaction incited by intact tissue transplants can be modified in several ways, so that it can be used only with considerable care in an analysis of problems pertaining to the development of species specificity.

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1940

JULY 9

Interrelations between egg-nucleus, sperm-nucleus and cytoplasm of the Asterias egg. Robert Chambers and Edward L. Chambers.

Asterias eggs removed from the ovary are in the germinal vesicle stage. Normal maturation occurs within an hour in sea water. Germinal vesicle eggs were cut into nucleated and non-nucleated fragments. These and whole eggs, after fading of the germinal vesicle, were inseminated at intervals before and after polar body formation.

The results obtained were as follows: (1) Insemination before polar body formation accelerates the budding off of the polar bodies by about 10 minutes; (2) The sperm aster never appears until after the second polar body has formed, irrespective of the insemination time; (3) The egg undergoes three distinct maturation phases after the germinal vesicle has faded.

During the first phase (20 to 60 minutes) the sperm nucleus lies quiescent in the egg cytoplasm. During the second phase (60 to 70 minutes) the sperm nucleus develops very slowly. The third phase extends from 70 minutes (shortly after first polar body formation) during which the sperm-nucleus develops at maximal speed.

The series of events may be summarized as follows: The spermatozoön enters the egg and hastens the maturation of the cytoplasm. The cytoplasm reaches complete maturation shortly before the formation of the first polar body. The matured cytoplasm simultaneously allows both egg-nucleus and cytoplasm to start development—on the one hand the polar bodies are pinched off, on the other hand the sperm-aster develops. In the insemination experiments with the nucleated and non-nucleated fragments of the germinal-vesicle egg it was found that the sperm-aster in the non-nucleated fragment develops slightly but constantly earlier than it does in the nucleated fragment. The delay in the nucleated fragment is associated with the polar body formation. It is likely, therefore, that the egg-nucleus, while it is active in forming polar bodies exerts a delaying action on the activity of the sperm-nucleus in forming the sperm-aster.

Digestion studies on salivary chromosomes. Daniel Mazia.

The relationship of protein and nucleic acid in the structure of salivary chromosomes of *Drosophila*, *Chironomus*, and *Sciara* has been investigated by the use of enzymes. Caspersson has shown that trypsin brings about disintegration of the achromatic regions. In the present work, nucleases were applied, and the history of the nucleic acid components was followed by means of Feulgen's reaction and by photography in the ultraviolet. Experiments with mixed nucleases demonstrated that the Feulgen staining material may be digested away without subsequent disintegration of the chromosome, which could still be demonstrated by the ninhydrin reaction. Photographs taken with light of wave-length 2650A indicated that the pyrimidine bases also were removed by this treatment. Comparison of individual nucleases indicated that nucleotidase (alkaline phosphatase of intestine), would alone produce the results described. This is interpreted to mean that the nucleic acid is attached to the protein of the chromosome through the phosphoric acid residues of nucleotides, as commonly assumed. These results contradict the theory of Wrinch. The problem of fibre formation by highly basic proteins has been attacked in the present investigation, and it has been found that thymus histone may easily be formed into fibres by compression of monomolecular films, although protamine does not form fibres when so treated.

Some properties of the residue from rapidly disintegrated Arbacia egg cytoplasm. M. J. Kopac.

Unfertilized *Arbacia* eggs were disintegrated in 0.53M KCl-solution at pH 6.8 to 7. Pigment and oil vacuoles, and granules were separated by centrifuging. Surface activities of the non-granular residue at oil-water interfaces were determined by: (1) measuring interfacial tensions with the flow-pressure method [Kopac, Colloquium on Micrurgy and Germ Free Technique, University of Notre Dame, in press] and (2) the estimation of interfacial adsorption by employing the drop-retraction technique [*Biol. Bull.*, **75**: 372, 1938].

The tension-lowering, Δ, is represented by the fraction, T_x/T_o, where T_o is the tension (dynes/cm.) at an *oil-water* [*0.53M KCl*] interface, and T_x is the tension at the *oil-water* [*0.53M KCl + residue*] interface. For cottonseed oil, $\Delta = 5.7/12.0 = 0.48$; for oleic acid, $\Delta = 0.6/7.5 = 0.08$. Similar values have been measured at *oil-protoplasm* interfaces using these oil phases and intact *Arbacia* egg cytoplasm.

The fraction of the total surface of an oil drop which is coated by surface-active molecules (proteins, protein complexes) within a given time is indicated by the adsorption ratio, δ. With cottonseed oil, δ equals 0.1 at 30 seconds and reaches a steady value of 0.2 in 10 minutes, while with oleic acid δ equals 0.9+ at 10 seconds and increases to 1 in less than 2 minutes.

The marked difference in tension-lowering of egg residue at cottonseed oil or oleic acid interfaces may be explained qualitatively by the difference in the amounts of proteins adsorbed by these oils. Accordingly, oleic acid showing the greatest adsorption also yields a lower interfacial tension when brought in contact with the residue.

By combining the values, Δ and δ, as obtained from flow-pressure and drop-retraction measurements, it is possible to relate the tension-lowering to adsorption. The following expression as developed from preliminary determinations agrees reasonably well with the data: $\Delta = T_x/T_o = n(1 - \delta)\epsilon^{-\delta}$. The correspondence between the observed and calculated tension-lowering at cottonseed oil interfaces is close ($n = 1$). However, with oleic acid the tension-lowering as calculated from δ is lower than the observed values ($n = 1+$ to 7). The values for δ are obtained from 10 to 30 seconds after the flow-pressure is recorded and one may therefore expect a greater adsorption at the time δ is measured particularly if the rate of adsorption is high. Also the elastic tension of adsorbed films of protein on oleic acid surfaces increases the flow-pressure so that the resulting interfacial tension values are higher than those expected on the basis of adsorption (from δ).

JULY 16

Ion intake by living cells. S. C. Brooks.

The present work is in marked contrast with the previously accepted conclusions as to the rate of movement of ions through the plasma membrane and the cytoplasm. These older conclusions were based on measurements of the total amount of ions in cells. Radioactive ions tell another story. When ions are transformed into heavier isotopes, e.g. Na_{11}^{24} instead of Na_{11}^{23}, they disintegrate and emit radiation, beta and gamma, which can be detected by very sensitive devices such as the Geiger-Müller counter. To obtain salts with activities high enough to be read and too low to injure cells, it is necessary to activate only one-billionth of the ions in the preparation. Under these conditions, it is considered that the concentration of the salt is essentially proportional to this radioactivity.

Cells are put into an excess of a dilute solution (0.0005M for Na_2HPO_4 to 0.033M for RbCl) in fresh or sea water or other normal habitat, according to the material. If the plasma membrane were rather impermeable to ions, it would be expected that active ions would be excluded. But these ions distribute themselves in a statistical equilibrium within an hour or two or in seconds, involving inorganic ion exchange. *Nitella* cells adjust themselves in about one minute for Na^+, K^+, Rb^+, and Br^-;

Spirogyra in less than 15 seconds; *Amoeba proteus* in less than 7 minutes, *Arbacia* eggs in 3–10 minutes for HPO_4^- and Na^+, and other marine eggs and sperm, and a yeast were tried with essentially similar results. This means that these cells are very permeable to ions, the rates observed being about 10^{-7} to 10^{-4} G.M. cm.$^{-3}$ sec.$^{-1}$, in contrast with 10^{-9} to 10^{-8}, the earlier supposition.

Change in salt concentration of the immersion fluid produces results in accord with the ideas that: (1) equilibrium is attained with salts present free and ions occupying attachment points in protoplasmic constituents; (2) the entering ions replace all intracellular ions in proportion to their own concentration and the replaceability of the intracellular ions.

Fresh water cells, e.g. *Nitella*, do not easily give up active ions to distilled water, but do lose them in a few minutes to inactive salt solution. This seems to show that ions enter independently, cations in relation to acidic groups in the protoplasm and anions in relation to basic groups. These groups constitute an effective mosaic membrane, as suggested by earlier workers.

Later stages in ion intake are complicated with losses of salts, and primary accumulation. These are shown in cells sacrificed for each observation, and in cells kept intact through a series of observations. In the case of *Nitella*, the latter is possible since the sap does not participate in this ion exchange, thus showing low permeability of the vacuolar membrane. These losses of salts, thought of as loss of ion pairs, rather than by ion exchange, and primary accumulation, are connected with metabolism. This may mean that metabolically produced organic ions are normally exchanged for entering inorganic ions.

The use of radioactive tracers in the determination of irreciprocal permeability of biological membranes. L. I. Katzin.

The study of the work done by frog skin on the cations passing through it, exhibited in the phenomenon of "irreciprocal permeability," has been hampered by the low permeability of the skin, and the necessity of using similar solutions on both sides of the membrane. By the use of radioactive "tagged" ions (Na^{24}, K^{42}), it has been possible to make quantitative measurements over a range of mixtures of KCl and NaCl (total chloride always 0.12 N), using chemically similar solutions on both sides of the skin. It has been found that a marked irreciprocality exists for the passage of sodium ion, the rate inwards varying from 60 per cent (pure NaCl) to over 300 per cent (20 per cent NaCl/80 per cent KCl) greater than the rate in the opposite direction. This corresponds to an irreciprocality of about $12–20 \times 10^{-8}$ gram ions per hour per square centimeter of membrane surface.

It has been suggested by Steinbach (*J. Cell. Comp. Physiol.*, **10**: 51 (1937)) that an excretion of potassium chloride through the skin may occur. This is tentatively confirmed for the lower ranges of potassium concentration. The reservation is based upon an uncertainty in the interpretation of the amount of radioactive salt retained by the skin itself, which is of the order of magnitude to balance the irreciprocality value. The difference in rate of passage in the two directions across the skin is about $2–6 \times 10^{-8}$ gram ions per hour per square centimeter in the outwards direction.

Urethane and the respiration of yeast cells. Kenneth C. Fisher and J. Stern.

The inhibition of oxygen consumption in yeast cells by ethyl urethane has been examined. Since the inhibitor must operate by combining chemically or by adsorption with some portion of the cell, it seemed possible that the effect of different concentrations of the narcotic should be related to the concentration by an expression derived from the law of mass action. On plotting so as to test this hypothesis, the data imply that there are two respiratory systems which add together to make up the

normal oxygen consumption. These two systems become apparent when the preparation is treated with urethane because they are affected by different concentration ranges of this substance.

It is found that the concentration of urethane which just blocks cell division in these yeast cells closely corresponds to the concentration which just suffices to inhibit completely the oxygen-consuming system having the higher affinity for urethane. Similarly, the data of van Schommerberg on luminous bacteria suggest that in that organism also the respiration is composed of two fractions, the ability to produce light appearing to be associated with the system which is affected by low concentrations of urethane.

It thus seems that in these two cells the respiration is composed of two distinct fractions which can be characterized by their quantitative relations with urethane. Furthermore, there is a close parallel between the effect of the narcotic on the respiratory system which is more sensitive to inhibitor and its effect on cell division and light production.

Spectrophotometric determinations on hemoglobin and its derivatives. Matilda Moldenhauer Brooks.

Curves obtained by the spectrophotometer and the microphotometer with infrared Eastman plates at wave lengths from 11,000 to 6,000 mμ show again that methylene blue does not change the absorption maximum of fresh sheep blood. This indicates that no methemoglobin is produced. $NaNO_2$ produces methemoglobin, but the addition of KCN to methemoglobin in the concentrations and conditions used in clinical medicine does not change the absorption maximum.

The entire explanation of cyanide poisoning and its treatment is based upon the relative oxidation-reduction potentials produced in the respiratory enzyme (cytochrome oxidase). This enzyme contains a heme group with a reversible system, producing a shift in the $Fe^{++} \rightleftharpoons Fe^{+++}$. This reversibility is destroyed by cyanide not because the cyanide unites with the Fe^{+++} as is generally assumed, but rather because the KCN produces a low redox potential poising the system at this level so that most of the Fe remains in the bivalent form and can no longer be oxidized. The respiratory enzyme can only act at a definite positive potential and ceases to function when this potential becomes sufficiently negative and respiration stops. This appears to be the mechanism of inactivation by cyanide, by analogy with experiments on hemoglobin.

To produce recovery, it is only necessary to add a substance producing a positive potential. $NaNO_2$ or methemoglobin itself added will do this so that the Fe of the enzyme can again function at its proper potential. The *production of methemoglobin by $NaNO_2$ is a by-product and does not enter into the mechanism.* When methylene blue is used, not only is the potential poised at a higher level, but the dye can take the place of the respiratory enzyme by virtue of its catalytic property, as stated by the writer in 1932.

Finally a shift in the absorption band of hemoglobin has been reported by some investigators using a hand spectroscope, when KCN is added to methemoglobin in certain concentrations. In this case an absorption maximum at wave length 555 mμ appears. This is the absorption maximum of reduced hemoglobin and indicates that it is identical with it, rather than that it is a new substance known as "cyanmethemoglobin." This change fits in with the theory that the negative potential produced by adding sufficient KCN produces a valence change in the Fe of the heme radical from Fe^{+++} to Fe^{++} without necessarily combining with it.

JULY 23

An in vitro analysis of the organization of the eye-forming area in the early chick blastoderm. Nelson J. Spratt, Jr.

When a piece containing the entire eye-forming area in the ectoderm plus underlying mesoderm and endoderm (Clarke, 1936) is isolated from the chick blastoderm

at either the definitive primitive streak, head-process, head-fold, or early somite stages of development and cultivated upon the surface of a blood plasma clot *in vitro*. it forms, as a rule, a fore-brain with optic vesicles or cups of rather normal structure, The isolate is thus shown to have the capacity for developing a morphologically organized structure of a specific sort. The fact that isolates from the older blastoderms give this result more frequently than do comparable isolates from younger ones indicates a change in organization of the eye-specific area.

Isolates containing anterior and posterior parts, right and left halves, and fourths of the area produce, in general, corresponding parts of the fore-brain, e.g., either an anterior or a posterior portion, or a right or a left half. Such isolates from younger blastoderms show a greater tendency to regulate toward the whole fore-brain than do those from older blastoderms. The development of these isolates indicates a regional localization or specification within the area which becomes progressively more stable during development.

When a blastoderm from which a piece just large enough to contain the eye-specific area has been excised is explanted on the surface of a clot, the excised area is replaced by endodermal, mesodermal and ectodermal cells surrounding it. A node-like structure and primitive pit may then arise in the regenerated region (in some cases). Subsequently, medullary plate and neural folds form, and finally a complete fore-brain with eye vesicles develop in many cases. Regenerative capacity is greatest during primitive streak stages, markedly decreased in head-process stages, and apparently lost as somites begin to form. Since the regenerated region undergoes the same kind of morphogenesis that a normal eye-forming area undergoes, it is inferred that an eye-forming area has been reconstituted.

On the determination of the vascular pattern of the brain of the opossum. Ernst Scharrer.

In mammals there exist two types of cerebral vascular patterns: In the one, found so far in all Placentalia, the capillaries form an unending network; in the other, discovered by Wislocki and Campbell (1937) in the opossum, an artery and a vein are always associated in a pair and the capillaries do not anastomose but end in hairpin-like loops. The question to be studied concerns the factors that determine the type of vascular pattern. These factors can be sought in peculiarities of the chemical or physical constitution of the living brain (Wislocki, 1939), or they may be regarded as inherent in the cerebral vascular system. The influence exerted by the living brain on the angioblastic tissue was tested in experiments in which pieces of dead, formol-fixed brains from rats and guinea pigs whose brains are vascularized by networks, were implanted into living opossum's brain which is supplied by terminal arteries ending in capillary loops. After 3 to 4 months the dead brain tissue is invaded by bloodvessels regenerating from the surrounding living brain and the pia. The vessels penetrating rat's or guinea pig's brain are of the opossum type. Accordingly in the reverse experiment, when dead opossum's brain is implanted into living rat's or guinea pig's brain, no capillary loops are induced, but a network grows from the host's brain into the implanted dead tissue. From these observations it is concluded that under the conditions of regeneration the characteristic vascular pattern of the opossum's brain is not forced upon the angioblastic tissue by the peculiar chemical or structural constitution of the living nervous tissue of the opossum's brain, but appears to be determined by factors inherent in the vascular system.

Functional properties of transplanted and deranged parts of the amphibian nervous system. Paul Weiss.

Part of the experiments have been briefly reported in *Proc. Soc. f. Exp. Biol. and Med.*, vol. 44, p. 350, 1940. Additional data were obtained to show that structurally deranged nerve centers exhibit synchronized rhythmic endogenous activity. Even minced nerve centers upon recovery produce such activity. Transplanted reflex arcs

break down and give way to generalized activity. In all cases reflexes are of mass character and involve the whole isolated center without finer localization. It has been shown that the method of "deplantation" used in these experiments is potentially of the same value to physiology as tissue culture has been in the study of morphological problems.

JULY 30

A study of feather color patterns produced by grafting melanophores during embryonic development. B. H. Willier and Mary E. Rawles.

Melanoblasts (from neural crest) of various breeds or species of birds grafted into the right wing-bud of white and pigmented fowl embryos (host and donor each 70 hours or equivalent age) migrate into the host feather germs, producing an area of donor specific coloration or pattern in the down and later in the juvenile contour feathers of the wing and adjacent regions.

Melanophores from pigmented donors deposit melanin granules of specific size, shape and color into the epidermal cells of the feathers, which are structurally of host origin. Melanophores from white breeds likewise enter and occupy all available positions in the feather germs, thus excluding those of the host (pigmented breed) which come in later. Since these contain a lethal factor, they die before depositing melanin, with the result that the host feathers are white.

The color or color pattern produced in the host feather is specifically in accord with the genotypic constitution of the donor melanophore. Thus, barred-rock melanophores produce a barred pattern in non-barred host breeds (N.H. Red, White Leghorn, and Black Minorca). Melanophores from a female donor (1 gene for barring) produce a darker-colored host feather than those from a male donor (2 genes for barring). The sex of the host has no effect on the result. Similarly, melanophores from male and female embryos (sex of donor ascertained after hatching) of F_1 hybrids (R.I. Red ♂ × Barred Plymouth Rock ♀) showing sex-linked differences in plumage produce, respectively, barred and non-barred contour feathers in a white leghorn host irrespective of its sex.

Although the melanophore behaves to a large degree independently, its action is under the control of extrinsic factors. For example, the width of the black bar produced by barred melanophores in non-barred hosts varies with the rate of growth of the feather. Similarly, guinea melanophores produce in white leghorn feathers color patterns practically identical with those of corresponding guinea feathers. Thus the guinea melanophore in a particular feather produces a specific color pattern. Each feather germ apparently has certain physiological properties (rate of growth, threshold of reaction, etc.) peculiar to it, which controls the action of the melanophore in pattern formation.

The cellular basis of the color pattern in some Bermuda coral reef fish. (Illustrated with color photomicrographs.) H. B. Goodrich.

The observations presented were made in Bermuda during the summer of 1939. Four species of parrot fish and one wrasse were studied. The cell structures of the dermis overlying the scales were interpreted by means of a series of stereograms. In the dark-green parrot fish, *Sparisoma viride*, the dermis shows layers of chromatophores, iridocytes and next to the scale a thick stratum of loose connective tissue. Throughout the connective tissue are found intercellular blue pigment bodies. In *Sparisoma abildgaardi*, the red parrot fish, there are very abundant erythrophores in the belly region. There are also present numerous translucent intercellular bodies of unknown significance. Other parrot fish studied were *Sparisoma squalidum*, *Scarus vetula*, and *Scarus caeruleus*. The last two named species also showed an abundant blue pigment, in some cases diffusely distributed. The wrasse studied was

the bluehead, *Thalassoma bifasciatum*, in which the blue color is due to refraction of light. Slides were also shown of color-producing elements in *Holocentrus ascensionis* and in *Atherina harringtoniensis*. The paper was illustrated with about 50 kodachrome lantern slides, some taken by reflected light and others by transmitted light and at various magnifications including the use of the oil-immersion lens.

AUGUST 6

Production of a complex nitrogenous compound, related to tyrosine, by a species of Penicillium. A. E. Oxford.

Although the lower fungi show certain biochemical resemblances to the algae, especially with respect to their carbohydrate metabolism and in the production of the sugar alcohols, mannitol and erythritol, no peptides corresponding to those isolated by Haas and Hill (*Biochem. J.*, **25**: 1472 (1931); **27**: 1801 (1933); **32**: 2129 (1938)), from marine algae have so far been isolated from mold tissue. Since the latter contains dipeptidase and a variety of polypeptidases (see Johnson and Peterson, *J. Bact.*, **29**: 90 (1935)), the presence of appropriate substrates might reasonably be inferred. In the course of investigations on the carbohydrate metabolism of *Penicillium griseo-fulvum* (see Raistrick *et al.*, *Biochem. J.*, **25**: 39 (1931); **27**: 628 (1933); **29**: 1102 (1935); **33**: 240 (1939)), a crystalline and weakly acidic compound, of empirical formula $C_{22}H_{32}O_8N_2$, and m.p. 172°, has been encountered, the structure of which appears to be derived from that of an acylated tyrosine. The medium on which the mold was grown contained glucose and sodium nitrate as sole sources of carbon and nitrogen respectively, and the yield of the above product was relatively considerable, accounting for 5–10 per cent of the nitrogen supplied as nitrate. A partial structural formula can be deduced from the following facts: acid hydrolysis yields a terpene-like hydrocarbon $C_{10}H_{16}$, together with NH_3, CO_2 (2 mols.), acetaldehyde, and the known base *p*-hydroxy-ω-aminoacetophenone. Alkaline hydrolysis of the metabolic product yields NH_3 (1 mol.), and a crystalline acid $C_{17}H_{22}O_3$, which is split by acid hydrolysis to yield the hydrocarbon $C_{10}H_{16}$ and *p*-hydroxybenzoic acid. The metabolic product appears, therefore, to contain a β-ketotyramine residue etherified with an alcohol $C_{10}H_{17}OH$, and linked probably through a peptide linkage to a residue yielding acetaldehyde on hydrolysis. The molecule probably contains an acid amide group also and the following structural formula is tentatively suggested:

$$C_{10}H_{17}\cdot O\cdot C_6H_4\cdot CO\cdot CH(CONH_2)\cdot NH\cdot CO\cdot C_2H_3O.$$

It is noteworthy that the mold in question yields a great variety of non-nitrogenous phenolic metabolic products in addition to the above, suggesting a possible connection between its carbohydrate and its nitrogen metabolism.

Studies on erythrocruorin (invertebrate hemoglobin). Kurt Salomon.

(This work will be published shortly in the *Jour. Biol. Chem.*)

Photochemical spectrum of the Pasteur enzyme. Kurt G. Stern, Joseph L. Melnick and Delafield DuBois.

(A preliminary communication concerning this work has appeared in *Science* **91**: 436, May, 1940.)

AUGUST 13

Effects of ultra-violet light on respiration of the luminous bacteria. A. C. Giese.

Luminous bacteria suspended in buffered glucose solution were irradiated in quartz Warburg vessels with a Sterilamp which emits most of its radiations at

λ2537A. The irradiated bacteria show only a very slight increase in respiration and luminescence immediately after irradiation. After a lapse of time the irradiated bacteria show a decline in respiration which is proportional to dosage and indicates that either the concentration of the nutrient or of the enzymes has been reduced. Experiments demonstrated that the radiations affect the bacteria directly, not by altering the nutrients. The decline in the respiration and the apparent decrease in the effective enzyme concentration is proportional to the dosage and after irradiation is stopped, this decrease does not continue, for bacteria irradiated in salt solutions to which glucose is added at intervals for as long as nine hours after irradiation show comparable respiratory rates following each addition of glucose.

Irradiated bacteria are similar to controls in that they respond to peptone to a comparable degree and are affected by urethane and cyanide in a similar manner, but they differ from the controls strikingly in their constructive activities, for their respiration declines much more rapidly, indicating their inability to replace components necessary for maintaining a given rate of respiration. Such bacteria are also unable to reproduce for colony formation may be prevented in most of the bacteria without altering the rate of oxygen consumption and dosages which reduce respiration injure the bacteria to such an extent that less than one in a thousand form colonies.

When extracts obtained from bacteria injured by ultraviolet radiations were added to suspensions of bacteria containing no nutrient, a marked increase in respiration occurred; when glucose was present, a much smaller increase was observed; when both glucose and peptone were present, and the respiration was probably near a maximum value, the extract had no effect. The extract thus appears to act as a nutrient, not as an accelerator.

Effects of ether upon the development of Drosophila melanogaster. Ivor Cornman.

Eggs of various ages were exposed for 20 minutes to an atmosphere ⅓ ether by volume. This dose is in excess of that used for anesthesia of the flies, and was chosen to give a clear-cut ether effect. In the series most extensively studied, the eggs were 5 to 20 minutes old at the beginning of etherization. The effects produced were of three types.

1: Cytological Abnormalities. The embryos showed abnormal spindles and fused or scattered chromosomes. Giant nuclei and multipolar spindles with polyploid numbers of chromosomes appeared, but no embryo was uniformly polyploid. The cytoplasm was also affected, becoming distributed in abnormal patterns. Complete disorganization showed in eggs which had not hatched, where undifferentiated masses of cells and non-cellular cytoplasm were found.

2: Developmental Abnormalities. Mortality, as judged by hatching, was 40.5 per cent as against the 8.3 per cent mortality of the controls. The mean hatching time was 21.29 ± .10 hours as against 19.79 ± .06 hours for the controls, a delay of 7.6 per cent. Mortality and rate of development were affected adversely in the larval and pupal periods as well.

3: Phenotypic Abnormalities. Deformation of the abdominal segments in a manner similar to the mutation *Abnormal abdomen* showed in 15 per cent of the emerged adults. The incidence of this phenocopy appears to be less when older eggs are etherized.

Ether, then, can produce visible abnormalities in the early embryo. These, or other changes, result in slower development or death in the embryonic, larval, and pupal periods, and produce abnormal adults.

Neurosecretory cells in cockroaches. Berta Scharrer.

Neurosecretory cells, i.e. cells which in addition to their nervous character show histological features of gland cells, are known in vertebrates as well as in inverte-

brates. Several species of cockroaches, as representatives of the insects, are suitable objects to demonstrate to what extent a nerve cell can assume the character of a gland cell. Different types of neuroglandular elements within one species suggest different phases of a secretory cycle. These stages are in principle similar to those observed in vertebrates. There is a stage when only fine fuchsinophile granules are scattered over the cytoplasm. The cytoplasmic inclusions appear to increase in size and number and may fill the cell to such an extent as to impart to it the character of a gland cell rather than that of a nerve cell. Such granules are also seen to extend from the cell along the axis cylinder. Finally there are cells giving the impression of an endstage in the cycle.

The morphological evidence of secretion in the central nervous system of insects is of particular interest in view of the physiological results obtained in recent years, which proved that the central nervous ganglia exert an endocrine control over the processes of molting (Hemiptera) and pupation (Lepidoptera). By means of transplantation experiments in *Rhodnius*, Wigglesworth recently succeeded in localizing the molting hormone in the dorsal half of the central mass of the brain, i.e. the very region where in the same species neurosecretory cells are found. There is good evidence to suggest, therefore, that glandlike nerve cells are actually the source of hormones which control insect development. This is the first case in which the morphological evidence for a neurosecretory activity can be corroborated by physiological data.

The mechanism of the glass electrode. G. Haugaard.

The glass electrode is of interest to the biologist for two principal reasons. Primarily, the glass electrode has become an important tool for the determination of pH. Secondly, experiments on the glass electrode itself have interest in relation to biological membrane phenomena.

Cremer, publishing the first paper on the glass electrode in 1906, was concerned only with this second aspect, namely its use as a model to elucidate certain bioelectric phenomena. The pH scale was unknown at that time.

The most satisfactory glass for the preparation of the glass electrode is that developed by MacInnes and Dole. Therefore this has been used in the present experiments. By electrolysis experiments it is shown that the sodium ion alone is responsible for the passage of electric current through the glass membrane. When a glass electrode membrane is prepared so that one surface has been soaked in water for a long time to establish an equilibrium, the other side never having been in contact with water, the reaction of the "fresh" surface with water may be studied uncomplicated by reverse effects. Under this condition there is a quantitative relation between the sodium-hydrogen exchange and the potential alteration of the system.

Experiments comparing the uptake of hydrogen ions and water by MacInnes and Dole glass powder show that the ratio of absorbed hydrogen ions to absorbed water is a constant, hence the absorbed hydrogen ions are solvated. In alcoholic solutions it could be shown that the hydrogen ions also carry alcohol.

On the basis of the above experiments, the following picture can be given of what happens when a fresh glass electrode comes in contact with an acid, neutral or weakly basic solution (i.e. within the range where the glass electrode acts only as a hydrogen electrode). At first the glass electrode will take up water and the sodium salt of the silicic acid will dissociate under the influence of this water. Hydrogen ion at the same time is absorbed. In other words, the sodium salt of the weak silicic acid is partially hydrolyzed at the surface, forming in the surface layer a skeleton of silicic acid. The solvated hydrogen ions react readily with the surface, which affords an easy entrance for the hydrogen ions into the glass. In the middle of the glass membrane there remains a layer of intact sodium salt. This theory is an extension of a theory developed by MacInnes and Belcher and also of an earlier theory by Horowitz.

AUGUST 20

Developmental changes in apical meristems. W. Gordon Whaley.

Apical meristems of several angiosperms were studied developmentally with respect to their rôles as functional embryos. The meristems were found to change greatly during development, the character and extent of the alterations being correlated directly with physiological differences. In longitudinal section the shape of the apical meristem changes from an almost flat structure in the seed to a strongly arched one in the mature plant. Volume of the meristematic region increases during the period of exponential growth, then becomes constant. Cell number follows essentially the same curve. Cell volume decreases progressively because the rate of division exceeds the rate of growth. The nuclei decrease in volume at a rate about two-thirds as rapid as the cells. As a result, there is relatively little cytoplasm surrounding the nuclei in the meristematic cells of mature plants.

Within a species or race a direct correlation was found between the size of the meristem and the size of the determinate organs which it produced. This correlation suggests that organ size inheritance in plants may be referred to the inheritance of meristem size.

The inadequacy of both Hanstein's three histogen (dermatogen, periblem, and plerome) and Schmidt's tunica-corpus system of meristem tissue designation was pointed out. The former resulted from an attempt to designate a specific 'germ' layer at the meristem while the latter fails to take into consideration several readily demonstrable differences between the epidermal layer and those layers immediately beneath it in plants with more than one tunica layer.

The relation between the four-carbon acid respiratory system and the growth of oat seedlings. Harry G. Albaum and Barry Commoner.

It was the purpose of this work to investigate the relation between the four-carbon dicarboxylic acid respiratory system and the various effects of auxin on oat seedlings. The effects of adding auxin and fumarate (which increase the activity of this system) and iodoacetate (which poisons this system) to the growing plants were determined.

The growth of the coleoptile is stimulated by the auxin in the seedling itself. When plants (of the variety Fulghum) were grown in various concentrations of iodoacetate the coleoptile growth was inhibited, the highest concentrations (.00005 to .0001 M.) resulting in a final size of but 50 per cent normal. The addition of auxin, and to a greater extent, of fumarate, negated the iodoacetate poisoning.

In contrast to the coleoptile, the growth of oat roots is known to be inhibited by the presence of applied auxin (10 mg. per liter). In the presence of iodoacetate this inhibition was partially removed, and conversely the inhibition was greatly magnified in the presence of fumarate.

Root number, which like the coleoptile length gives a positive response to this concentration of auxin, behaved like the coleoptile toward iodoacetate and fumarate.

It has been suggested by Thimann that all organs show a similar response to auxin, the direction of the effect being a function of auxin concentration. Thus, low concentrations stimulate, higher concentrations give an optimum plateau, and even greater concentrations inhibit. The different effect of the same auxin concentration on different organs is accounted for by the displacement of each of these curves along the auxin concentration axis, and also by the intrinsic auxin present in the particular species or variety. By testing the effect of various concentrations of iodoacetate on these phenomena, we have been able to confirm and extend this interpretation. When iodoacetate and auxin concentration are plotted in opposite directions on the same abscissa, and effect on the ordinate, it is possible to produce the hypothetical curves relating effect to active auxin concentration.

Respiratory changes following stimulation in Nitella. R. K. Skow and L. R. Blinks.

The large and very slow electrical disturbance following stimulation in *Nitella* is known to have so many of the properties of the nerve impulse that it seemed desirable to follow the changes in respiration during and after its passage down the cell. Oxygen consumption was measured in Schmitt's modification of the Fenn respirometer, using a travelling microscope on a micrometer screw mounting to follow the movement of the kerosene index droplet. The resting respiration of the cell (0.015–0.02 mm.^{3} O_2 per minute) was increased 50 per cent to 100 per cent during repeated electrical stimulation (once per minute). By careful attention to temperature control it was also found possible to measure the changes following a *single* stimulation. An increase of 20 per cent or 30 per cent in O_2 consumption followed for some 10 or 15 minutes after a single propagated action current, gradually returning to the resting rate. Much smaller increases followed action currents restricted to only part of the cell; there was no increase on repeated sub-threshold stimulations, nor any volume change on continued flow of much larger currents through a dead cell.

A frequent characteristic of the respiratory response was a temporary *decrease* of the rate of movement of the index drop for about 5 minutes following stimulation, before the increase appeared. This was not a temperature artifact, but could represent either a momentarily decreased respiration rate, or an R.Q. temporarily greater than unity (the extra volume of CO_2 being a little too slowly absorbed by the KOH).

Partially in order to clarify this effect, an independent method of following CO_2 production was employed, using a thin film of $Ba(OH)_2$ on a filter paper strip brought very close to the cell, in a closed vessel of small volume. The electrical resistance rise of this strip during precipitation of the barium carbonate was followed in a bridge circuit. A marked increase of CO_2 production followed *immediately* after a single stimulation with this method, indicating no temporary decrease of CO_2 production. Whether ammonia production is possibly involved in the initial counter movement in the volumeter is still to be answered.

Neither irritability nor its accompanying excess CO_2 production could be abolished within periods up to 24 hours in purified hydrogen.

The relation of potassium to the bioelectric effects of temperature and light in Valonia. L. R. Blinks.

Damon has demonstrated the influence of KCl concentration upon the bioelectric potential of *Valonia*, while Marsh has described certain large temperature effects. The author has attempted to correlate these. Repetition of temperature studies with a great many cells (in sea water) shows a curious concave curve, with a minimum P.D. between 20° and 25° C., a sharp rise on warming between 30° and 35°, and a slower, though regular rise on cooling to 15°. (Cooling to 8 or 10° C. again lowers the potential, sometimes with poor recovery.)

The magnitude of the potassium effect (P.D. change on halving, doubling or quadrupling the KCl content of sea water) closely parallels the potential value in normal sea water at these temperatures. It is smallest at 25°, with the typical cusp and later fall of P.D. described by Damon at room temperature; at 15° it becomes flat-topped and appreciably larger; while at 35° the cusp is followed by a rapid rise to higher values. These differences may be due to the speed with which KCl actually enters the protoplasm, abolishing the experimentally altered gradient across the outer surface, and finally reaching the vacuolar surface, according to Damon's scheme.

Cells were also exposed to temperature changes while continuously bathed with sea waters of different K content. The temperature effect was almost abolished in 0.006 M KCl or lower; was normal at 0.012 M; and became considerably enhanced

in 0.024 and 0.048 M KCl. Almost exactly the same results were found with the effects of light (ascribed by Marsh to oxidation-reduction potential changes in the protoplasm). In K-free sea water there is no light effect, or sometimes a reversed one; in 0.006 M a slight effect; in 0.012 M the normal one; while in 0.024 or 0.048 M considerably increased effects. Evidently potassium closely controls the magnitude of these effects, probably *via* its altered entrance and accumulation under metabolic or other influences.

General Scientific Meetings

August 27

The hydrogen ion and the osmotic concentrations of the cytoplasm in Vorticella similis (Stokes) as indicated by observations on the food vacuoles. S. O. Mast and W. J. Bowen.

The food vacuoles are formed by inward pressure of the cilia in the gullet. They are spindle-shaped and formation requires 50± seconds. During this period much fluid passes out, resulting in marked concentration of the solid particles in suspension.

After leaving the gullet they become spherical, decrease greatly in size (2± minutes) and their contents become acid (pH 3±). Two minutes later they increase very rapidly and greatly in size and their contents become nearly neutral (pH 6.9±).

The changes in the H-ion concentration in the food vacuoles were ascertained by observations on ingested yeast cells which had been stained by boiling them in solutions containing respectively Congo red, brom thymol blue and brom phenol blue.

The acid in the vacuoles is produced in the wall of the gullet and the cilia in it and condensed in the vacuoles, owing to differential permeability of the surface membrane. It is neutralized by rapid entrance of cytoplasmic fluid which is approximately pH 6.9.

The ingested bacteria die 16 seconds after the vacuoles leave the gullet when the acidity is pH 5±. Death may be due to this acidity.

If the concentration of the culture fluid is increased by 0.025 M (0.625 atmospheres) or more the body decreases in size. The osmotic concentration of the culture fluid used equals 0.79 atmospheres. That of the cytoplasm therefore equals 1.385 atmospheres. This doubtless varies directly with the concentration of the culture fluid owing to adaptation.

The decrease in the size of the vacuoles varies inversely with the concentration of the fluid in them, *i.e.*, the culture fluid, but they still decrease in culture fluid which is so concentrated that the entire body decreases in size and the osmotic concentration in them is higher than that of the fluid in the cytoplasm. This is due to inward pressure of the stretched membrane at the surface of the vacuoles.

The reversibility of certain artificially induced changes in the permeability of the erythrocyte. M. H. Jacobs and W. D. Jones.

The senior author and his associates have previously reported the production of striking changes in the permeability of the erythrocyte by narcotics, heavy metals (copper and mercury), salicylates, and especially by pH changes. The earlier results were obtained chiefly by the use of the hemolysis method which, while convenient and quantitative, may be complicated by factors not related to permeability. Much of this work has therefore been repeated, using the photoelectric method of Parpart to record the volume changes of the cells that occur when a small amount of a penetrating substance such as glycerol is suddenly added to a stirred suspension of the cells in isotonic NaCl. The results obtained by this entirely independent method

confirm very satisfactorily the earlier conclusions. In addition, both methods give evidence of a rather high degree of reversibility of most of the changes previously described. The permeability of human erythrocytes to glycerol, after being greatly reduced by M/4 ethyl urethane, may be restored to its original value by removing them to a urethane-free solution; and these changes can be repeated at least two or three times in succession. The greatly decreased permeability of human erythrocytes to glycerol produced by 10^{-5} M $CuSO_4$ may likewise be reversed in the same manner, or more readily and rapidly by the addition to the copper-containing cell-suspension of a very small quantity of serum or of hemolyzed cells. At a concentration of 10^{-5} M, $HgCl_2$ produces similar reversible effects. The greatly reduced permeability of human erythrocytes to glycerol at pH 5.0 or 5.5 may also be almost instantaneously restored to its normal value by adding enough alkali to bring the pH of the solution to 7.0 or above. On the other hand, the enormous reduction of permeability to NH_4Cl produced by low concentrations of tannic acid has so far proved to be reversible only to an inconsiderable extent by the methods used.

Oxidase activity and respiration of cells and cell-fragments. E. J. Boell, R. Chambers, E. A. Glancy and K. G. Stern.

The rate of respiration and the ability to oxidize *p*-phenylene diamine of mature *Arbacia* eggs is greater than that of immature *Arbacia* eggs. When the mature eggs are fragmented by high-speed centrifuging, the addition of *p*-phenylene diamine to the light 'halves,' containing the female pronucleus produces a larger increase in the oxygen uptake than is the case with the heavy 'halves' containing the granules. The 'disintegrum' obtained by shaking eggs in a calcium-free medium shows oxidase activity but no respiration.

The rates of respiration of mature and immature *Asterias* eggs are of the same order of magnitude. *P*-phenylene diamine and methylene blue, when added to immature starfish eggs, produce approximately the same increase in the oxygen uptake, the effects of the two substances being additive in character.

An attempt was also made to study the enzymatic activity of the large nuclei of the salivary gland of *Chironomus* larvae. The whole gland shows a definite respiration which is somewhat increased by glucose, pyruvate or *p*-phenylene diamine. Treatment of the glands with dilute egg albumin, at low temperature, abolishes the respiration but does not appreciably affect the oxidase system. Nuclei, isolated by this maceration procedure, appear to possess oxidase activity.

Respiratory metabolism of mating types in Paramecium calkinsi. Edgar J. Boell and Lorande L. Woodruff.

Respiratory metabolism of two mating types of *Paramecium calkinsi* has been measured by means of the Cartesian diver technique in order to determine whether a physiological basis for the difference in mating behavior between the types can be discovered.

In animals in which the mating reaction consists of 'agglutination' with subsequent pairing, the average rate of oxygen consumption per animal per hour is 0.25 mμl for Type I and 0.28 mμl for Type II. This difference possibly does not represent a real type difference in metabolic rate since the average size of Type II animals is slightly greater than that of Type I.

The oxygen consumption of animals in which neither agglutination nor pairing occurs is much larger than when both of these phases are manifest. The average respiratory rates for Type I and Type II animals are then, respectively, 0.43 mμl and 0.48 mμl per animal per hour.

Occasionally, the mating reaction consists only of the agglutinative phase without subsequent pairing. In such cases, the individuals of one type show the low rate of oxygen consumption characteristic of *Paramecia* which will both agglutinate and pair,

whereas the individuals of the other type respire at the typically high rate of animals in which mating tendency is absent.

The results indicate that an inverse relationship exists between metabolic level and mating tendency and that agglutination without pairing will occur between animals in which one type possesses a high rate of metabolism while that of the other is low.

Squid ink, a study of its composition and enzymatic production. Eric G. Ball and Pauline A. Ramsdell.

Ink expressed from the ink-sac of *Loligo pealii* contained 38.9 per cent organic matter and 5.7 per cent inorganic matter. The black insoluble material of the ink could only be centrifuged down after the addition of 3 volumes of 95 per cent ethyl alcohol. Partial purification was effected by resuspension in water and reprecipitation with alcohol, this process being repeated twice. The precipitated ink was finally washed with 95 per cent alcohol and air dried. A yield of 26 grams of dry ink was obtained from 380 ink-sacs. Qualitative tests on this material showed Cu and N to be present, Fe and S to be absent. It contained about 6 per cent ash, an amount which was not appreciably diminished by prolonged dialysis.

The ink-sac was the only tissue of the squid which yielded extracts capable of catalyzing the air oxidation of dihydroxyphenyl-alanine. Active enzyme preparations were obtained by grinding the sac with sand and 0.1 M Na_2HPO_4, centrifuging, and precipitating the enzyme from the black supernatant by the addition of an equal volume of saturated ammonium sulfate. The precipitate collected by centrifuging was dissolved in water, a black insoluble material removed and the enzyme precipitated by stepwise fractionation with $(NH_4)_2SO_4$. The fraction precipitated between 35–40 per cent saturation showed the most activity. About 300 mg. of such material was obtained from 380 ink-sacs. The protein precipitate of this fraction yielded colorless solutions and catalyzed actively the air oxidation of dihydroxyphenyl-alanine and catechol but not tyrosine. Dialysis against water inactivated the enzyme. Activity was not restored by addition of a concentrate of the dialysate. The activity of the enzyme was completely inhibited by diethyldithiocarbamate at a concentration of 1.3×10^{-4} molar. Other less effective copper poisons listed in the order of their effectiveness were K dithiooxalate, K ethyl xanthate, 8 hydroxyquinoline, and K ferrocyanide. Cyanide also inhibited the enzyme. Analysis of the enzyme preparation showed it to contain 0.033 per cent copper. It is concluded that the ink-sac of the squid contains a copper protein compound capable of catalyzing the air oxidation of orthohydroxyquinones. Hemocyanin, the copper blood pigment of the animal, possessed no such catalytic activity.

Observations on the occurrence of simple ethereal sulphates in marine algae. A. E. Oxford.

The micro-volumetric method devised by Øllgaard (*Biochem. Z.*, **274**: 181 (1934)) for the determination of inorganic sulphate has been modified so that the SO_4 of polysaccharide sulphuric esters may be determined simultaneously. After acidification of the liquid containing both inorganic sulphate (not more than 0.25 mg. S as SO_4^{--} per cc.) and ethereal sulphates with an equal volume of 25 per cent trichloroacetic acid, polysaccharides are precipitated by addition of 4 volumes of acetone, and to the supernatant is added water and finally a concentrated solution of benzidine in acetone in order that the final concentration of acetone may be 71 per cent by volume. The polysaccharide precipitate is hydrolysed by boiling N. HCl and the liberated SO_4^{--} precipitated as benzidine sulphate as in Øllgaard's method. Similarly, the total SO_4 (inorganic and ethereal) in the original liquid is determined after hydrolysis.

Cold-water extracts of numerous freshly gathered marine algae have been

examined by the above method, and in a few instances, notably *Dasya pedicellata* (C. Agardh) C. Agardh among the Rhodophyceae and various species of *Ulva* among the Chlorophyceae, the presence of polysaccharide sulphuric esters as well as inorganic sulphate has been demonstrated. The polysaccharides cannot be of great complexity since, once redissolved in water, they cannot be reprecipitated by the addition of alcohol or acetone. One specimen of *Ulva Lactuca* Linnaeus v. *latissima* undoubtedly contained a still less complex ethereal sulphate which could not be precipitated at all by acetone. Such compounds are of interest in that they may be regarded as intermediates between the SO_4 ion and the very complex polysaccharide esters of sulphuric acid such as agar-agar and the polysaccharide from *Chondrus crispus*.

Blood-sugar and the problem of the pancreas in lampreys. E. J. W. Barrington.

Previous work by the present author and by others has suggested that the zymogen component of the vertebrate pancreas may be represented in the ammocoete larva by an accumulation of secretory cells of a special type in the intestinal epithelium at the anterior end of the mid-gut, this region having a tendency to grow out into blind caeca in both larvae and adults of certain species. It has been known for many years that during the life of the larva there are budded off from the same region of the epithelium groups of cells which form gland-like follicles lying in the submucosal connective tissue, and it has been suggested more than once, on purely histological grounds, that these might represent the islet tissue of higher forms; if this could be definitely established, the lampreys would seem to exhibit an interesting early stage in the formation of the pancreas. The hypothesis has been tested in preliminary experiments by destroying these follicles in the ammocoete larva by cautery, and comparing the blood-sugar of the operated animals with that of animals upon which a control operation had been carried out. In a group of eighteen of the former, the mean blood-sugar value (measured 48 hours after the operation) was found to be 110 mgm. per cent, while in a group of twenty controls the value was 35 mgm. per cent. The difference is statistically very highly significant, and while clearly not in itself a proof of the existence of an insular mechanism, it provides the first physiological support for that suggestion.

Fatty acid compounds in the unfertilized egg of Arbacia punctulata. Albert E. Navez and Arthur B. DuBois.

Using the technique described in a previous note[1] based on extraction with cyclohexane after removal of water by centrifugal packing and alcohol treatment, "crude oil" of *Arbacia* eggs was prepared and fractionated.

The attention was centered especially on the glycerids fraction, reserving for later (after preliminary examination) the study of the small quantities of sterols, phospholipids and free fatty acids present in the crude oil. In the present work the red pigment mentioned in the previous note was removed by adsorption on norite before any further proceeding, in view of the fact that it seems to act as an oxidation catalyst for the glycerids. These are built up from saturated fatty acids and from unsaturated ones and both types have been recovered by Farnsteiner's lead salts method. The unsaturated fatty acids have been separated in the form of their bromine derivates and also by Tsujimoto's lithium salt-acetone solubility method. The mono-, di-, tri- and tetraethylenic acids are present; the relative proportion of each one is not constant throughout the season and does not seem to vary according to any obvious or definite rule. The same can be said about the relative proportion of saturated acids to the unsaturated although as a rule the quantity of unsaturated acids appears larger at the beginning of the season than at the end of the summer. All these variations are decidedly larger than the experimental variation.

[1] A. E. Navez, *Biol. Bull.*, 77: 323, 1939.

The glycerids extracted and the fatty acids separated show marked spontaneous oxidation. By measurement of the oxygen consumption of thin films it is possible to show that the oxidation proceeds at possibly three different rates. After a short induction period the oxygen fixation proceeds at a relatively high rate for a lapse of time, then slows down to a new level for a period then again slows up further for a long period.

It seems probable also from investigation of the ease with which the oil can be extracted that the fatty compounds can and do exist in the cytoplasm in three forms:

(1) The "granular oil" associated with the fatty granules dispersed in the cytoplasm, easily attacked even at room temperature, by such solvents as cyclohexane and removed by means of a short hot extraction.

(2) Some "oil" released from the cytoplasm or cytoplasmic structures from which it can be detached by centrifuging according to the method used by Harvey for stretching the egg without breaking it in two parts and allowing it to round itself up again. The actual amount of energy involved in the process of stretching seems small *a priori*, but it is large enough nevertheless to "split off" this portion. Perhaps one should keep in mind that the same procedure brings also differences in behavior of "stretched and rounded" eggs in other processes (NH_3 release, enzymes).

(3) After treatment of eggs with an ammonium salt according to Heilbrunn's observation, the oil cap of centrifuged eggs becomes increasingly larger as the ammonium treatment lengthens. After such a treatment with ammonium chloride, it is possible to extract by the method indicated here an additional quantity of the crude oil. To all appearances this oil existed in the cytoplasm as bound to proteins.

Work on the actual composition of the three portions of oil, now in progress, will be carried on in the future.

Distribution of plasmalogen in certain invertebrate forms. C. Giddings.

The method used for the determinations was similar to that suggested by Feulgen. The values obtained show an interesting distribution in various tissues of invertebrate forms, and represent milligrams plasmal per 100 grams fresh tissue. Excess moisture was removed from the tissues by gently pressing between filter papers. Thymonucleic acid, glucose, etc. do not interfere. Highest values were obtained from the gills of mollusks (260–320 mg. from *Modiolus* and *Busycon* gill), whereas, for example, the gills of *Cancer irroratus* showed only 23–44 mg. plasmal.

High values were also obtained from the gonad and kidney of mollusks, with lower values from liver tissue.

Muscular tissue in general was also relatively low in plasmal content (often less than 15 mg.). Low values were also obtained from typical sponges, as well as from *Bugula*, *Perophora*, *Pennaria*, *Amaroucium* and Protozoa.

Lipoids and their probable relation to melanophore activity. G. H. Parker.

The chief activators of the melanophores in the catfish *Ameiurus* are three: intermedin from the pituitary gland, acetylcholine from the dispersing autonomic nerves, and adrenaline from the concentrating autonomic nerves. Catfishes blanch under the influence of adrenaline and darken under that of intermedin and acetylcholine. Dark caudal bands are formed by the action of intermedin and acetylcholine. Such bands can be formed in hypophysectomized catfishes where acetylcholine is the only dispersing activator present and may persist two weeks. Acetylcholine which will be destroyed almost at once in the aqueous fluids of the catfish is soluble in oil and in this solvent will remain active in a catfish for days. Is the dispersed state of the melanophores in a dark caudal band due to acetylcholine stored temporarily in the fish's lipoids or to continued activity of the dispersing nerves whereby new acetylcholine is produced? When a cold-block is put on dispersing nerve-fibers and after fifteen minutes the fibers are cut ventral to the block, the area of the nerve

darkens from the cut to the block but not beyond. If after an interval of time the block is removed from the fish on which the test has been made and the fish is allowed to swim, the peripheral part of the nerve area will darken. This interval of time may be as long as six and a half hours and represents a period over which the nerve has been producing acetylcholine. How much longer this process might go on is unknown. Nor is it known whether in the days that may follow during which the caudal band exists, the band is kept up by new acetylcholine from the nerve or by old acetylcholine which may have been stored in the adjacent lipoids or by both.

Studies of the distribution of the autolytic mechanism. S. Belfer, B. Bailey, H. C. Bradley and H. Eder.

A survey covering a wide range of forms shows that all vertebrate tissues examined possess the same well-defined type of autolytic mechanism found in mammalian tissues. Cathepsin is the characteristic proteinase of the system, with an optimum in the neighborhood of pH 4. No digestion occurs at pH 2, and very little at 7.5. It appears to be as universally present in active metabolizing vertebrate tissue as is respiration itself, and is probably of fundamental importance in protein metabolism. It is believed to mediate the synthesis and cleavage of proteins in hypertrophy and growth, and in the protein mobilizations which occur in starvation, involution, atrophy and necrosis.

Invertebrate tissues present no such single autolytic pattern. There are many different enzyme mechanisms represented, just as there are various oxygen carriers in invertebrate bloods. In some tissues there is a generalized autolysis over a wide pH range. In some there is evidence of cathepsin. In still others there are enzymes of the pepsin and trypsin type, acting at pH 2 and at 7.5. Thus squid and *Pecten* muscle digest over a wide pH range, but to a very limited extent. A slight optimum appears at pH 4. It is unlikely that these animals can mobilize muscle proteins to tide them over a prolonged period of starvation as do the vertebrates.

In the pedal muscle and the albuminigerous gland of *Busycon*, there is no digestion at pH 4, a marked maximum at pH 2, and a second smaller one at 7.5. The hepato-pancreas of *Busycon* digests slowly between pH 7 and 5, rising sharply to a peak at pH 2. If cathepsin is present it is completely masked by the pepsin-like enzyme present. The hepato-pancreas of the lobster resembles vertebrate gland tissue and appears to contain cathepsin.

The effect of various cholinergic drugs on the radula protractor muscle of Busycon canaliculatum. Carl C. Smith.

The radula protractor muscle of *Busycon canaliculatum* is admirably suited to physiological and pharmacological study. Preparations suspended in sea water remain viable for 36 to 48 hours. It appears to be histologically unique, consisting, according to Dakin,[1] of a central sarcoplasm surrounded by contractile sarcostyles. By careful dissection one can obtain good nerve-muscle preparations.

The response to most drugs of the parasympathomimetic type is a typical contraction. In the case of acetylcholine, the minimal concentration is usually 1 : 5,000,000, and 1 : 10,000,000 after eserine 1 : 100,000 has been applied. Nicotine 1 : 165,000 causes a slight contraction, but this concentration has no visible effect on the normal contraction produced by a just effective addition of acetylcholine. The effects of acetylcholine (1 : 1,000,000) or carbamylcholine (1 : 500,000) are almost entirely antagonized by both novocaine and cocaine, though the same concentrations and novocaine and cocaine will, by themselves, produce no effect.

Curiously enough, potassium ions even up to a concentration of 0.3 per cent or 3 : 1,000, have no effect on the muscle; higher concentrations cause a slow prolonged

[1] Dakin, Wm. J., (1912) *Proc. Trans. Liverpool Biol. Soc.*, **26**: 253.

contraction quite distinct from that produced by the choline esters. The relative insensitivity to ergotamine and ergotoxine differentiates this tissue from typical vertebrate smooth muscle.

Choline esterase in nerve fibers. E. J. Boell and D. Nachmansohn.

Recent investigations suggested that choline esterase may be concentrated at or near the surface of nerve cells and that the increase of enzyme concentration to high values at synaptic regions may be connected with the increase of surface due to the "Endarborisation" of fibers at synapses.

Direct evidence has now been obtained for this assumption. By means of the Cartesian diver technique, the enzyme activity has been determined separately in the axoplasm and the sheath of the giant fiber of the squid. Practically the total enzyme activity is found in the sheath, whereas the amount of enzyme present in the axoplasm is negligible. The $Q_{CH.E.}$ (mg. acetylcholine split by 100 mg. fresh tissue in 60 min.) was, e.g., in one experiment 0.420 in the sheath and 0.027 in the axoplasm, that is 17 times higher in the sheath. This is a minimum value, since a large part of the sheath is connective tissue. The enzyme concentration may be actually much higher than the activity per unit of tissue weight indicates, because the enzyme may be localized in a small fraction of the remaining volume. The figures found for the activity of the total giant fiber are, as could be expected, intermediate between those for the sheath and those for the axoplasm.

These experiments indicate that a high concentration of the enzyme exists at or near the surface of the nerve cell. They support the suggestion made by one of us (D.N.) in conjunction with previous observations that the activity of nerve cells may be connected everywhere at or near their surface with the metabolism of acetylcholine and that the phenomenon may be only quantitatively more important at synapses owing to the great increase of the surface. This may explain the findings of Lorente de Nó that acetylcholine can be liberated from fibers as well as at synapses and is compatible with the conclusion of Gasser and Erlanger that conduction of nerve impulses along fibers and across synapses differs only quantitatively.

Vascular reactions to renin and angiotonin. Richard G. Abell and Irvine H. Page.

Arterioles, capillaries, and venules in transparent moat chambers in rabbits' ears were observed with the microscope following the intravenous injection of renin and angiotonin [Page and Helmer, *J. Exp. Med.*, **71**: 29–42 (1940)] and the effect of these substances upon the blood vessels and blood flow determined. Comparable studies were made with epinephrine.

The intravenous injection of 0.2 cc. of renin was followed by arteriolar contraction. At the time of greatest contraction, 2.8 minutes after the injection, the arteriole photographed had become 0.24 its original diameter. Contraction was not complete and did not interrupt the flow of blood in any of the vessels. The renin had no appreciable effect upon the diameters of the capillaries and venules.

The intravenous injection of 0.2 cc. of Ringer's solution was not followed by contraction of any of the vessels.

The intravenous injection of 1 cc. of angiotonin was followed by arteriolar contraction, which became greatest 2.5 minutes after the injection. At this time the arteriole photographed had decreased in size to 0.53 its original diameter. Contraction was not complete and the flow of blood was not interrupted in any of the vessels. The angiotonin had no appreciable effect upon the diameters of the capillaries and venules.

The intravenous injection of 0.05 mg. of epinephrine was followed by very extensive contraction of the arterioles. Contraction became greatest 4.3 minutes after the injection, at which time the lumen of the arteriole photographed had

decreased in size to 0.04 its original diameter. As a result of arteriolar contraction, the blood flow in the arterioles and capillaries stopped. Injection of epinephrine was followed also by contraction of the venules. At the time of greatest contraction, 7.9 minutes after the injection, the venule photographed became 0.33 its original diameter. The epinephrine had no effect upon the diameter of the capillaries.

Factors affecting the frequency of contraction of the heart of Venus mercenaria. Albert E. Navez, John D. Crawford and Dora Benedict.

In the note published in 1939[1] conditions governing the isotonic contraction of the isolated heart were studied and among them the aeration and the addition of 1 part of dextrose to 250,000 parts of sea water in the bathing fluid, the loading of the muscle and the temperature were recognized as being of primary importance. Under these conditions after an initial rise of the frequency curve from the moment of excision to a maximum, in about two hours, then dropping very slightly to a new level, this plateau level could be maintained constant to within less than 1 per cent for a period as long as 120 hours.

For the isometric contraction one can generally state that the same conditions prevail, although the frequency does not rise as high as under isotonic contraction and the period of constant frequency of contraction of the isolated heart is reduced to 15–20 hours. The maximum tension which can be set up by an average heart weighing 125 mg. (net weight) can reach under optimal conditions of temperature and circulation, 2600 mg.

The diagram tension-time is a curve rising (in 1.5 hrs.–2 hrs.) from a low value shortly after excision to a sharp maximum (where the tension reaches twice the initial value), then declines to an intermediate plateau value in 5 to 6 hours from excision time. The value is then maintained for 10–15 hours, followed by a period of gradual decline and ends with complete exhaustion of the heart.

The frequency-time curve is of the same order except for the much broader maximum and for the delay in reaching this maximum.

In order to determine energy exchanges, measurements of O_2 consumption during isotonic and isometric contractions have been made, the heart being under different loads and the load being applied at one end or both ends of the muscle serving as suspension points and the load being attached to the middle.

A very good correlation exists over a wide range of loads between oxygen consumption and loading. The maximum work obtainable during isometric contraction and the rate at which it is performed correlate very well with the values obtained for critical loading of the heart and the time relations found for isotonic contraction.

Determining factors in the regeneration of Hydractinia. Charles E. Wilde, Jr.

Hydractinia echinata, a polymorphic, unbranched, colonial hydroid, exhibits, under certain conditions, a change of polyp form on regeneration. In the control series of experiments, pieces of stalks of gonozooids were severed from the living substrate and cut below the sporosacs. In all cases, in regeneration, the pieces reconstituted the gonozooid form.

In series A individual gonozooids were isolated, having attached at the bases a small amount of the living substrate. Cuts were made in the same manner as in the controls. In 94 of 116 cases, the stumps regenerated as gastrozooids.

Series B was then run, in which the polyps were treated similarly to series A except that a slanting cut was made so that the regenerate incorporated a small portion of sporosac-bearing region. Fifty-five of 64 cases regenerated as gonozooids.

[1] A. E. Navez and J. D. Crawford, *Biol. Bull.*, 77: 315, 1939.

Gastrozooids were then isolated in the same manner. Cuts were made below the tentacular ring. In all cases the regenerates developed as gastrozooids.

Small pieces of sporosac region of gonozooids, stained with Nile blue sulphate, were grafted into the most oral tissues of gastrozooids cut below the tentacles. In 18 out of 90 cases the graft influenced the regenerate so that a gonozooid was formed, or a gonozooid and a gastrozooid.

A control seems to be exerted by definite regions of the polyps in regeneration. There is a gonozooid-influencing region in the most oral third of the gonozooid. The horny covered living substrate is the gastrozooid-influencing region. There appears to be a balance between the two regions, that which is dominant being expressed in the final form of the regenerate. The gonozooid influence seems to be the more potent per unit mass. Evidence for similar but less well-defined systems in *Hydractinia* dactylozooids awaits more data.

Time of determination and dominance in tubularian reconstitution. Edgar Zwilling.

An attempt has been made to study the process of reconstitution in *Tubularia* in such a manner that information might be obtained concerning such questions as to when the new hydranth is determined, and when the inhibiting effect of this new hydranth is exerted on lower levels of the stem. Young summer colonies of the hydroid were employed in the experiments reported. By the application of ligatures or small glass capillaries to the cut distal surface (a procedure which inhibits reconstitution when done immediately following section) at intervals, it was found that if the cut surface is exposed to running sea water for from 10 to 15 hours before the ligature or the cap is applied, then the hydranth would continue to develop despite the presence of the inhibitors. The hydranth may be considered as being "determined" after this interval. In addition, it was found that the number of proximal ends which reconstituted in these 10 mm. stems decreased as the number of distal hydranths increased. Further, when similar 10 mm. stems were ligatured in the middle at intervals after sectioning, it was found that the inhibition (dominance effect) of the proximal end by the distal was exerted during the latter part of the process—after at least 10 hours had elapsed. This seems to coincide with the time when the distal hydranth is determined. (The inhibition is evinced as a decrease in the number and size of the hydranths, and an increase in the time for formation.) It was also noted that the initial period of reconstitution was more susceptible to an inhibiting concentration of KCN. Stems placed in this solution after 5 hours exposure to sea water were much more inhibited than those placed in the KCN after 15 or 20 hours.

A regeneration-inhibiting substance released by Tubularia tissue. S. Meryl Rose.

If a small colony of *Tubularia crocea* is placed in 250 cc. of sea water and air bubbled through the water continuously, the solution becomes an inhibitor to regenerating stems. The degree of inhibition of regeneration is proportional to the length of time the inhibiting solution was in contact with the living colony. Four-hour colony water delayed regeneration approximately two and one-half hours in freshly cut 10 mm. stems. Twelve-hour colony water completely inhibited regeneration in most stems. More than 600 stems were observed in this experiment. Living adult hydranths also inhibit regeneration of hydranths. Even regenerating stems inhibit each other. Ten stems in 50 cc. of sea water regenerated more rapidly than either 20 or 30 stems in the same volume of sea water. This experiment was conducted eleven times with the same result every time. The degree of inhibition with crowding varied from a one-hour to a five-hour delay in the 30-stem group when compared with the 10-stem group.

The possibility that colony water and crowding inhibited regeneration because of oxygen deprivation or carbon dioxide accumulation was eliminated when the experiments were repeated in flasks through which air bubbled vigorously. Both the colony water and crowding effects were observed.

The inhibitor is thermally unstable. Heating the solution from room temperature to 80° C. and immediately cooling it destroys almost all of the inhibiting effect of twelve-hour colony water.

All of the experiments cited above were conducted between temperatures of 20° and 22° C. The crowding effect is scarcely observable at 18°. Other experiments have shown that ligatured stems do not commence to regenerate at temperatures between 19° and 23°. At temperatures from 14°–16° stems commence to regenerate even while ligatured in standing sea water. The coupling of these facts leads to the working hypothesis that the substance responsible for the crowding effect is produced in the tissue and must be removed before regeneration can occur. It is considered probable that the release of this substance at a perisarc-free surface accounts for regeneration at that point.

The rôle of oxygen in regeneration of Tubularia. L. G. Barth.

Experiments in which the oxygen concentration was varied between 100 per cent and .3 per cent showed that the oxygen consumption varied from 100 per cent to 29 per cent and the rate of regeneration from 100 per cent to zero. Further analysis shows that the time for regeneration is not affected in the higher oxygen concentrations from 10 to 100 per cent but that the size of the regenerant varies. At lower oxygen tensions both size and time were affected.

Other experiments showed a two-fold effect of oxygen. Nitrogenated sea water inhibits regeneration if the stems are placed in it immediately after cutting. If, however, the stems are first treated with oxygenated sea water for varying lengths of time and then placed in nitrogenated water, they will regenerate, the rate of regeneration increasing as the time of exposure to oxygenated sea water increases. A short exposure of 2 hours to oxygen delays regeneration but with 5 hours or more exposure, the time for regeneration becomes fixed and only the size of the regenerant varies.

From the experiments it is concluded that the effect of oxygen is two-fold. First there is a 5-hour activation period during which the time for the process of regeneration is determined. Second, the continual application of oxygen allows more and more cells to become involved in the formation of a hydranth.

The growth of oat coleoptiles after seed exposure to different oxygen concentrations. Harry G. Albaum.

The growth of *Avena* coleoptiles may be modified after grains have been soaked for 20 hours in solutions of glucose and 3-indole acetic acid as well as in water at different temperatures (Albaum, Kaiser and Eichel, in press). All these agents in the concentrations used lowered or raised the oxygen content of the soaking fluids. Within the limits of the experiments, the following relations obtained: the lower the oxygen content, the slower the velocity of growth, the longer the growth period and the larger the final coleoptile length.

The present experiments were performed in order to ascertain whether the same kind of relationships obtained when the oxygen content of the soaking fluids was varied more widely with oxygen-nitrogen mixtures.

Grains were soaked for 20 hours in water through which oxygen-nitrogen mixtures containing 0, 2.5, 5, 10, 20, 50 and 100 per cent oxygen were bubbled. After the treatment the grains were planted in beakers in contact with filter paper and the growth of the coleoptiles was recorded at regular intervals. The reciprocal of the time required to attain half final length was used as an index of rate of growth.

In addition, measurements of oxygen consumption were made with a Warburg respirometer at the close of the soaking period.

As the oxygen concentration was increased between 2.5 and 20 per cent, final coleoptile length decreased. Correlated with this decrease, there was an increase in rate of growth. In the 50 per cent mixture, final length and rate of growth both decreased. Respiratory rate, on the other hand, increased with increasing oxygen concentration up to 50 per cent. At 100 per cent final coleoptile length was inhibited markedly (84 per cent) as compared to the 20 per cent mixture, although rate of respiration and rate of growth were inhibited only to a relatively small extent (17–19 per cent). The last observation is of particular interest since it represents another case where growth may be markedly inhibited without bringing about a proportional decrease in respiration.

Preliminary experiments aerating grains in water for 0, 4, 8, 12, and 24 hours showed the effective period for altering growth rate and final size to occur some time after 8 hours.

Results of transplantation of the pituitary anlage to the thyroid region in Amblystoma. W. Gardner Lynn.

It has recently been demonstrated by Etkin (1939) that, in the frog, transplantation of either the pituitary or thyroid anlage in such a way as to bring the two developing glands in close proximity to each other results in precocious activation of the thyroid. This activation is strikingly evidenced by metamorphic precocity as well as by the histological picture presented by the thyroid. The evidence indicates the existence of some previously unknown thyrotropic influence emanating from the early pituitary, which is of the nature of a field effect rather than a hormone.

The present experiments were originally conceived as an attempt to ascertain whether this effect is concerned with the rate of differentiation of the activated gland. A series of 150 operations was performed on *Amblystoma* at Harrison's Stage 29. In all cases the pituitary anlage of one animal was transplanted to the thyroid region of another of the same stage. Donor, host and control were raised in small Stender dishes, duplicate series being kept at 10° and 25° C. The animals were preserved at intervals ranging from 4 hours to 25 days after operation and some were kept as long as three months. Stages were thus available ranging from the origin of the thyroid gland from the pharynx to well-differentiated glands with actively secreting follicles. Study of the sectioned material reveals no significant difference in the degree of development or activity of the thyroids of the control and experimental animals at any stage studied. Since in nearly all cases the transplanted pituitaries were found very near the thyroid and in many cases were in actual contact with it, and since the results in *Rana* under such conditions are so unequivocal, the failure of *Amblystoma* to show the effect must indicate that the urodeles differ markedly from the anurans with respect to this feature.

August 28

Oxygen consumption of Arbacia eggs following exposure to Roentgen radiation. T. C. Evans.

Irradiation of either sperm or ova does not suppress the rapid increase in oxygen consumption which accompanies fertilization. Preliminary data indicate a probable relationship between level of oxygen consumption rate and rate of development. The rate of oxygen consumption, after the initial stimulation, appears to be higher in the control lots than in those irradiated with dosages which reduce the cleavage rate.

Effects of roentgen radiation on the jelly of the Arbacia egg. I. Disintegration of the jelly. T. C. Evans.

The results of experiments conducted this summer indicate that Roentgen radiation (at 5900 r/m.) has a direct effect on the jelly which normally surrounds the egg.

The radiation causes the immediate disappearance of the jelly from around the egg. Eggs placed in irradiated sea water or in water from irradiated eggs retain the jelly. The jelly is affected even in buffered solutions. The presence of the jelly in a solution of egg-water can be demonstrated by staining with Janus green. An irradiation of 59,000 r or more results in a negative test for the presence of jelly in the egg water. The power of agglutinating spermatozoa is greatly reduced in the irradiated egg-water.

Effects of Roentgen radiation on the jelly of the Arbacia egg. II. Changes in pH of egg media. By Marshall E. Smith and T. C. Evans.

Irradiation of sea water with dosages of 59,000 r does not significantly alter the pH. *Arbacia* eggs in sea water or in an iso-osmotic NaCl-KCl mixture irradiated with the above dosage produced a small but definite shift of the pH of the solution towards the acid side (pH 7.4 to a pH of 7.2 by a dosage of 25,000 r). When eggs were irradiated in solutions buffered at pH 7.4 this value remained unchanged.

Irradiation on jelly alone in the NaCl-KCl solution (59,000 r) reduced the pH from 7.4 to 7.0. The pH of the jelly solution was not altered when irradiated in a medium buffered at pH 7.4 with 0.01 M. glycylglycine. The jelly was disintegrated, by sufficient radiation, in both buffered and unbuffered solutions.

When eggs were cytolyzed by shaking in distilled water the pH was lowered to 5.5–5.9 and was not significantly affected by radiation.

A pH of 4.9 or lower was found sufficient to remove the jelly without any irradiation. There was little loss of jelly when the solution had as high a pH as 10.0.

It is concluded that irradiation by disintegrating the jelly and injuring the egg results in a lowering of the pH of the egg water.

Delay in first cleavage of Arbacia eggs following Roentgen irradiation of zygotes. E. P. Little and T. C. Evans.

The delay in time of first cleavage produced by irradiating the zygote immediately after fertilization yields a dose-effect curve which approximates a logarithmic character (as has been reported by previous investigators).

The character of the dose-effect curve can apparently be altered by changing the conditions of the experiment. Such factors, which appear to alter the dose-effect relationship are: (1) time of irradiation after fertilization, (2) intensity (if extreme) of the radiation, and (3) developmental conditions during and after the irradiation.

Concerning sensitivity of cells to X-ray. Grace Townsend.

Calcium release or ionic imbalance theories do not adequately explain the "fixation" of injury by fertilization (Henshaw, 1939) or gradient pattern sensitivity of embryos (Hinrichs, 1926). These may find interpretation in the postulate that X-Rays affect SS : SH ratio in the cell as determining potential held "fixed" as a mechanism of development. Kammerling (1937) and others report SH freed by irradiation.

Polarity of organisms is believed determined in the egg; multipolar cleavage in *Arbacia* (X-ray effect, faulty polarity?) is followed by faulty polarity of embryos. Oxygen tension and H-ion concentration may determine polarity of *Fucus* eggs, and the same factors affect SS : SH ratio (Voegtlin, Green). Sperm path may determine

polarity, I found tested sperm of high SH content. Polarity was studied by Child largely by relative reduction capacity of regions which appears to be related to free SH groups (Lund, 1931, Green, 1933, Maluf, 1933).

I found $Na_2S_2O_4$, glutathione, methylene blue, and ascorbic acid modified polarity of *Arbacia* embryos in relation to reduction potentials. X-ray effects were simulated by appropriate concentrations of glutathione: (a) in *Nereis* eggs activation and abnormal elevation of membranes, (b) in *Arbacia* delayed cleavage, exaggerated fertilization cones, multipolar cleavage, modified polarity of embryos, and "fixation" of injury by fertilization. Added glutathione sensitized *Arbacia* eggs to X-ray (3,500–7,000 r) resulting in cleavage delay and distorted polarity (by appearance) greater than the sum of the effects of the separate agents, also interfered with recovery.

Tumor tissue has a high SH and SS content and is relatively sensitive to X-ray. I found pea seedlings, soaked seeds, growing tips, and *Nereis* eggs of much higher glutathione concentration than the respective materials of comparison found to be far less sensitive to X-ray (Henshaw, 1933; Bruno, 1939).

The above and other phenomena (latent effects, high sensitivity of nucleus, etc.) cannot be solely explained by inorganic theories but could be interpreted by the postulate suggested.

Laboratory ripening of Arbacia in winter. Grace Townsend.

Twelve dozen sea-urchins distributed in four aquaria in two experiments were ripened Oct. 20–Dec. 20 and Dec. 21–Jan. 23 respectively. The temperature was regulated to near 18° C.–19° C. with occasional fluctuation. Algae, clams, and hard-boiled hen's eggs were the principal foods given though other marine forms were acceptable. Artificial light was supplied in the first experiment, but it was found in the second experiment that ripening occurred in total darkness as well as with continuous illumination. After one month's ripening October 18, dredged sea-urchins had normal appearing gonads but the females were frequently with oöcytes. After two month's ripening all of ten females yielded normal appearing ripe eggs which stratified normally when centrifuged and with a cleavage time of 55–70 min. at 21°–22° C. Eggs from summer urchins left standing had a cleavage time of 75–120 min. In the first experiment the gonad centrifuged volume from six specimen samples increased from 6.5 cc. to 12.9 cc. or 16.8 cc. for two experimental tanks with light, heat, and food supplied while controls with temperature unregulated regressed from 6.5 cc. to 3.4 cc. Summer ripened urchins left standing regressed to a sample volume of 2.2 cc. In the second experiment in one month of ripening the six specimen gonad sample volumes increased from 6.2 cc. to 8.0 cc.–8.9 cc. with light and to 10.0–11.8 cc. in darkness.

Occasional starfish and sand-dollar specimens with fertilizable eggs were observed in late December. *Nereis virens* spawns in March or April. The possibility of a winter supply of marine eggs at the laboratory is suggested.

A note on determining the sex of Arbacia punctulata. Ethel Browne Harvey.

Inject a drop of sea water saturated with KCl into one genital pore by means of a very fine hypodermic needle (No. 27). The eggs or sperm will almost immediately begin to ooze out from this pore alone, and the sex can be determined by the color (red ♀, white ♂). The shedding is stopped at once by placing the animal in a jar of still sea water.

Centrifugal speed and the Arbacia egg. Ethel Browne Harvey.

The size of the two "halves" into which the *Arbacia punctulata* egg is broken by centrifugal force varies with the speed. With a low speed of the electric centrifuge

(4,000 × g), the white half is twice the volume of the red. With the highest speed of the electric centrifuge (10,000 × g), the white half is 1½ times the volume of the red. With a low speed of the air turbine (50,000 × g), the halves are of equal volume. With the highest speed of the air turbine (100,000 × g), the red half is five times the volume of the white.

There is a difference also in the packing of the granules. With low speeds, the granules are well packed before the egg breaks in two; with high speeds, the egg breaks before the granules are well packed.

If the large red half obtained with high speed is re-centrifuged (10,000 × g), it stratifies like the whole egg but there is no nucleus. These large halves obtained with the air turbine develop much better than those obtained with the electric centrifuge, both the fertilized and the parthenogenetic merogones.

Colored photographs of stratified Arbacia punctulata eggs stained with vital dyes. Ethel Browne Harvey.

The different layers of the egg stratified by centrifugal force are stained differentially with different vital dyes, nicely shown with Kodochrome films. The jelly surrounding the egg is stained with Janus green (purple) and toluidin blue (pinkish lavender). The oil and nucleus are not stained. The clear layer is slightly stained with Nile blue (blue), toluidin blue (purple), rhodamine (pink), neutral red (pinkish yellow), chrysoidin (yellow) and Bismark brown (yellow). The mitochondrial layer is stained differentially with Janus green (blue), methyl green (purple) and gentian violet (purple). Yolk and pigment are stained blue with methylene blue, brilliant cresyl blue and Nile blue; purple with toluidin blue and methyl violet; red with rhodamine and neutral red; yellow or brown with chrysoidin and Bismark brown. With saffranin, the pigment is stained blood red, the yolk is unstained.

Elongation and return in spherical cells. Herbert Shapiro.[1]

When unfertilized *Arbacia punctulata* eggs are elongated into the dumbbell shape by centrifuging, and then transferred immediately to sea water, the course of return to normal spherical shape can be observed and measured microscopically. There is an initial rapid decrease in length, followed by a much slower, almost asymptotic approach to the spherical condition. Cells washed in calcium-free artificial sea water and then centrifuged, both elongate farther and round up more quickly than those kept in sea water or artificial sea water. Increasing the strength of calcium in the artificial sea water, while holding pH and osmotic pressure constant, results in cells rounding up more slowly than in normal sea water. These effects may be due either to alterations in surface forces, or the protoplasmic viscosity or both. According to current theory, the chief forces governing the division of a cell are its metabolism, surface conditions, and viscous properties. If one or more of these variables are altered as in these experiments, then the conditions imposed above should have an effect on the speed of cleavage. Unfertilized eggs washed thoroughly in sea water freed of calcium by the addition of a stoichiometric quantity of sodium oxalate and removal of the precipitate, go through first cleavage more rapidly than cells in sea water. This was observed by treating both sets of eggs identically, fertilizing them at the same time, agitating them gently in a water bath at constant temperature, and removing samples at five-minute intervals for fixation and subsequent counts of percentage cleaved. The complete curves of percentage cleavage against time after fertilization were then plotted. Calcium-free artificial sea water gave results similar to oxalated sea water. Artificial sea water with excess calcium was found, on the other hand, to retard cleavage, as compared with controls in normal artificial sea water.

[1] Aided in part by a grant from the Penrose fund of the American Philosophical Society.

Echinochrome as the sperm activating agent in egg water. Ivor Cornman.

It has long been known that eggs of various marine invertebrates produce substances which stimulate sperm, but the nature of the substance has never been determined. Recently Hartmann and others announced that echinochrome, the pigment which gives *Arbacia* eggs their reddish color, stimulates the activity of *Arbacia pustulosa* sperm. However, in experiments begun last summer at Dr. Eric Ball's suggestion, no stimulation of *A. punctulata* sperm by echinochrome could be detected.

Further experiments were carried out this summer under carefully controlled conditions. Most important is control of pH, since sperm are inactivated by weak acids and reactivated by bases. To eliminate any activation by pH difference, all media were held at the same pH by glycyl-glycine and piperazine buffers. The solutions of crystalline echinochrome were tested immediately as to their activating effect before any loss of the echinochrome by precipitation as a calcium salt, or by decomposition in the more alkaline solutions could take place. Several test series were carried out with suspensions at pH 6 for sea-water and pH 7 for isotonic NaCl solutions, in which the sperm were nearly motionless, but were readily activated by egg-water of the same pH. Under the same conditions echinochrome did not activate, in concentrations ranging from 1 : 25,000 (more deeply colored than egg-water) to 1 : 1,000,000,000. Other series carried out at higher pH values gave similar results. Clearly echinochrome is not the agent in *A. punctulata* egg-water which activates the sperm.

Tyler also reports no effect of echinochrome on *Strongylocentrotus* sperm activity or oxygen consumption. The discrepancy may be due to species difference, or to the fact that in the work with *A. pustulosa*, acidity was not controlled, since pH is not mentioned in any of the papers. By reason of its limited occurrence in invertebrate species and its inactivity in *A. punctulata*, echinochrome cannot be ranked as a general sperm-activating agent.

A relation between the dilution medium and the survival of spermatozoa of Arbacia punctulata. Teru Hayashi.

Spermatozoa suspended in seminal fluid (testicular plasma) were found to retain activity and fertilizing power longer than sperm in equal concentration in sea water. Microscopic observation showed that the sperm were more intensely active in the seminal fluid than in the sea water. Therefore the assumption that the death of sperm is caused by the exhaustion of an internal source of energy did not seem to be applicable to these experimental results. pH did not seem to be an effective factor in the survival of the sperm.

Saturation of the seminal fluid with ammonium sulfate brought down a precipitate which was filtered out. An artificial seminal fluid was made by dialyzing the residue against sea water to a volume approximating the original seminal fluid sample. The ammonium sulfate was removed from the filtrate in the same manner. This filtrate dialysate thus consisted of the original seminal fluid minus the precipitated constituent. Sperm suspended in equal concentrations in these preparations were found to live longer in the dialysate containing the precipitate. However, this artificial seminal fluid was not so effective as the natural product in keeping the sperm functional.

This precipitated constituent of seminal fluid seemed to be heat-sensitive. It is concluded that protein in the seminal fluid seems to be effective in prolonging the survival of sperm. The nature of the reaction between the sperm and protein is not known. There are three possibilities to be investigated; a nutritive relationship, a surface adsorption, and a relationship involving colloidal osmotic pressure.

The occurrence of cartilage at the bifurcation of the common carotid artery in an adult dog. William H. F. Addison.

In examining serial sections of the carotid sinus and adjoining regions in a series of fifty dogs, the presence of cartilage was observed on both the right and left sides in a large adult animal. The nodule of cartilage was about the same shape and size on both sides. It had a rounded form and measured nearly a millimeter in diameter. It was situated between the external and internal carotid arteries, as they arise from the common carotid artery. In this situation the walls of the adjoining blood vessels are highly elastic in structure and the nodule of cartilage is also traversed by elastic fibers. The nodule of cartilage, by reason of its size and position, causes slight bulges in the adjoining walls of the carotid sinus and of the external carotid artery. Cartilage is constantly found in a similar position in the young horse and is later replaced by bone. The bilateral position of the cartilage at the bifurcation of the common carotid artery suggests that the cartilage is a derivative of a branchial arch.

Cytoplasmic morphology in the gizzard of Gallus domesticus. Hope Hibbard.

Although general descriptions of the histological structure of the gizzard are available in the literature, the accounts of its development are incomplete. In the 1880's Cazin, and in 1925 Conselius, traced its embryology, but both studies lack cytological precision.

The observations in this report on the secreting epithelium of the chicken gizzard confirm the earlier accounts of an original simple epithelium which later becomes thicker, and at regular intervals, downgrowths into the dermis occur (not upgrowths of the connective tissue into the epithelium as described by Cazin). Subsequently, in each of these columns of cells a lumen appears through which the keratinoid secretion pours up to the surface. This is not a cornification of a stratified squamous epithelium as Kaupp (1918) has said.

The "Golgi apparatus," demonstrated by silver nitrate or osmic acid, figures prominently in these cells, changing from elongated parallel rods to a complicated network. Its interpretation in terms of aqueous vacuoles seems reasonable after neutral red postvital staining. A considerable amount of negative evidence as to its composition has been accumulated. It is not fat or lipoid (evidence from frozen sections); it is not glycogen (negative to iodine after absolute alcohol fixation); it is not mucus (negative to mucicarmine); it is not keratohyalin (negative to the Pasini stain). There are chondriosomes to be found in its neighborhood which sometimes compose a part of the blackened net.

A majority opinion exists that these glands do not secrete enzymes although Iliine (1913) claims the presence of a diastase. A mucoid secretion of certain cells of the gland, a secretion of keratohyalin by others, and the gradual disintegration and sloughing off of those cells nearest the gizzard lumen to form part of the lining, were observed.

Papers Read by Title

Further studies of photodynamic action in the eggs of Nereis limbata. Fred W. Alsup.

Parthenogenetic development can be induced in *Nereis* eggs by photodynamic action. The best development was obtained in eggs exposed in solutions containing 1 part rose Bengal and 200,000 parts sea water to light from a 1000-watt bulb. In these parthenogenetically developing eggs, first and second polar body formation and first cleavage show the same time sequence as in inseminated eggs developing normally in sea water. Later cleavages are usually irregular, but many of the

parthenogenetic eggs give rise to ciliated larvae which swim slowly and show varying degrees of differentiation.

Germinal vesicle breakdown due to photodynamic action can be prevented by the removal of calcium from the eggs. Eggs which were immersed in isotonic solutions of potassium or sodium citrate for 10 minutes and were then removed to solutions of rose Bengal in isotonic potassium or sodium chloride, did not show any germinal vesicle breakdown when subjected to photodynamic action. However, if eggs were removed from the chloride solutions to sea water containing rose Bengal, their germinal vesicles were broken down by photodynamic action.

When eggs were removed from sea water to isotonic citrate solutions containing small amounts of rose Bengal and were placed in the dark, some of them became heavily stained and showed germinal vesicle breakdown. The dye and citrates are believed to act in unison to produce a lytic effect on the eggs.

Although no photodynamic effects can be produced on *Nereis* eggs in the absence of free oxygen, the effects produced on eggs by concentrated solutions of rose Bengal acting alone in the dark[1] can be obtained in the absence of free oxygen.

The eggs of some females showed a small percentage (1–3 per cent) of germinal vesicle breakdown when they were exposed in sea water to artificial light.

A quantitative study of the effect of cyanide and azide on carbonic anhydrase. C. W. J. Armstrong and Kenneth C. Fisher.

The effect of the oxidative inhibitors cyanide and azide on living tissues exposed to them can be described by the mass law (Fisher et al., *J.C.C.P.*, vol. 16, p. 1, 103, 1940). The proportionality factor "a" in the mass law equation, which indicates the ratio of inhibitor to enzyme during the reversible inactivation, is found to have values characteristic of the inhibitor and system used. For cyanide four separate values are suggested averaging 0.4, 0.8, 1.2 and 1.6, while for azide "a" is 1. In attempting to elucidate the significance of the fractional values for cyanide, it is important to have similar data on any isolated enzyme systems which are sensitive to these inhibitors. Such data have now been obtained for carbonic anhydrase.

The enzyme preparation used was the crude chloroform extract described by Meldrum and Roughton (*J. Physiol.*, vol. 80, p. 113, 1933) and its activity was estimated by the "boat" method described by those authors. The values obtained are tabulated below:

	Cyanide	Azide	Azide
Initial pH	6.7	6.7	5.7
Final pH	7.6	7.6	7.0
"a"	1.07, 1.16, 0.91*	1.08, 1.01	0.91, 1.07

* Calculated from the data of Kiese and Hastings (*J. Biol. Chem.*, vol. 132, p. 281, 1940).

The average "a" of each set of data differs from unity by an amount which is within the limits of reproducibility of the experiments.

Upon the sources in the insect head of substances which influence crustacean chromatophores. Frank A. Brown, Jr. and Alison Meglitsch.

The American cockroach, *Periplaneta americana*, was used to determine the source of substances in the insect head possessing chromatophorotropic properties. Using

[1] See Alsup, *Biol. Bull.*, 77: 324.

isolated pieces of integument of *Cambarus* bearing red and white chromatophores as the test material, it was found that the corpora cardiaca were the sources of a very powerful substance effecting concentration of crayfish red chromatophores. This tissue was still effective when extracted in more than 50,000,000 times its volume of salt solution. Qualitatively this gland was shown to differ from crustacean sinus gland in not possessing also a white pigment-dispersing action such as is found in the latter gland. Also, the rate of loss of effectiveness upon red pigment of dilution was shown to be much greater for corpora cardiaca than for sinus glands, suggesting a difference in the constitution of the red effecting principles of the two glands. Of the tissues tested, white pigment-concentrating material was found only in extracts of frontal ganglia and the brain while such material was absent from extracts of the thoracic ganglia. The corpora allata extracts possessed no chromatophorotropic activity.

Myofibrillar modifications in the caffeinized frog heart. Ralph H. Cheney.

The current studies present the microphysical modifications which appear in the striated myofibrillae of ventricular strips from *Rana pipiens* Schreber after immersion in caffeine. Two per centum caffeine-in-Ringer at pH 7.37 was used at room temperature for variable periods from three to thirty minutes. The material was fixed in Zenker's fluid and stained with phospho-tungstic acid-haematoxylin.

Normal controls and the caffeine experimental strips both showed a number of "spot disc" or local clot type areas as illustrated by Jordan (1912) and often involving only one or a few sarcomeres of one myofibril. An occasional transverse intercalated disc extending through a considerable number of myofibrillae of the muscle fiber was noted as described by Jordan (1912), who interpreted them as irreversible contraction bands. In addition, a variety of incipient or partial clot formations occur and seem to be identical with the microphysical changes due to congelation phenomena as induced by faradic current or chloroform in striated muscle by Nageotte (1937); by Speidel (1938 and 1939) by several types of stimulation in living skeletal muscle; and induced by caffeine immersion in the case of the frog gastrocnemius by Cheney (1939).

It is suggested that the sequence of physical changes in the myofibrillar shortening process is a constant in cardiac muscle as it is in skeletal muscle. However, a comparison of the degree of histo-physiological changes produced by caffeine upon striated cardiac tissue is never as drastic as similar concentrations for an equal time of immersion in the case of the striated skeletal muscle myofibrillae. Full retraction clots involving the complete disorganization of extensive myofibrillar areas with the formation of Zenker's wavy degeneration areas are absent. Even 2 per cent caffeine for a thirty-minute immersion period causes a degree of myofibrillar modification (injury) in frog cardiac muscle which is comparable to the effects of only a 0.03 per cent caffeine solution for an equal period in the instance of skeletal muscle. The cardiac myofibrillae are much less susceptible to caffeine action (injury threshold) than the striated muscle.

Effects of visible radiation on Arbacia eggs sensitized with rhodamine B. Leonard B. Clark.

Arbacia eggs sensitized by immersion in 1 : 25,000 solution of Rhodamine B in sea water and illuminated with light of 128,000 foot-candles before fertilization showed increase in time to secure 50 per cent cleavage, increase in number of eggs giving abnormal cleavage and increase in percentage of eggs undivided at the end of three hours after insemination and an increase in number of eggs cytolyzed with length of exposure.

By using the percentage of eggs remaining undivided three hours after fertiliza-

tion as a measure of the photic effect, the relation between effect and exposure yields a sigmoid curve with the inflexion point at 57 minutes exposure.

The mechanism by which light acts on the egg-dye complex is not clear, for eggs placed in radiated solutions of Rhodamine B show similar but less intense effects to eggs radiated in the dye. Eggs previously illuminated and placed in unradiated dye show no difference over controls placed in sea water.

Effects of cyanide on cleavage in eggs of Ilyanassa and Crepidula. A. C. Clement.

Uncleaved eggs of *Ilyanassa* were placed into solutions of KCN in sea water varying in strength from N/100 to N/20,000. In all of these cleavage progressed in apparently normal fashion for some time, except that in the stronger solutions, at least, the division rate was slower than in the controls. The observations were made on eggs which had been removed from their capsules and it should be mentioned that such eggs when kept in sea water did not survive beyond about 2 or 3 days and did not produce fully-formed larvae, although ciliated embryos were formed. In certain cases eggs in cyanide solution survived far longer than the controls, but it is doubtful whether in any of them differentiation progressed beyond the point reached by the controls. In N/100 KCN cleavage did not progress beyond about the 16-cell stage. In N/1000 KCN cleavage, though somewhat retarded, reached a fairly advanced stage, but then development appeared to cease even though the eggs survived for several days longer. In weaker cyanide solutions (N/5000, N/10,000, N/20,000) development progressed to the point of producing ciliated embryos, but eventually stopped.

Eggs of *Crepidula plana* gave a different result. Uncleaved eggs placed into N/1,000 or N/5,000 KCN were blocked but in most cases only after cleaving once or twice. On removal to sea water even after remaining in the original cyanide solution for as long as 3 days, further, though abnormal, cleavage occurred.

Thus the initial cleavage phase of development in *Ilyanassa* is rather insensitive to cyanide. This is in contrast to the situation in some other eggs—*Arbacia*, for example—where cleavage is readily blocked by cyanide. Conceivably, in *Ilyanassa* the energy for cleavage is supplied either through a cyanide-insensitive respiration, or through anaerobic processes. Evidence is not at hand to prove or disprove either of these conjectures.

The cell origin of the prototroch of Nereis limbata. D. P. Costello.

E. B. Wilson's study of the cell-lineage of *Nereis* (1892) established the fact that during normal development the prototroch originates from the four trochoblasts of the 16-cell stage, $1a^2$–$1d^2$ (Wilson's $a^{1.1}$–$d^{1.1}$). Each of the original trochoblasts, in two cleavages, gives rise to four cells, of which three remain in the prototroch, and the fourth lies above in the upper hemisphere of the larva. The twelve prototrochal cells put forth prototrochal cilia at about the tenth hour.

The study of the development of isolated blastomeres provides a means of verifying these observations and of ascertaining the capacity for self-differentiation of the isolated cells. After separation at the 2-cell stage, AB and CD continue to develop and each produces a partial prototroch at the proper time. When isolated at the 4-cell stage, A, B, C, and D, after continued cleavage, differentiate a ciliated prototrochal band. Isolating at the 8-cell stage, 1a, 1b, 1c, and 1d cleave and later differentiate prototrochal cilia, while 1A, 1B, 1C, and 1D do not become ciliated. When the blastomeres of the 16-cell stage are isolated, four cleave only twice and then differentiate prototrochal cilia. By progressive separation of blastomeres of a given egg at the first, second, third, and fourth cleavages it can be demonstrated that these four cells are the trochoblasts ($1a^2$–$1d^2$). In certain cases one may observe that three of the four division products of the trochoblast are ciliated. The apical cells

($1a^1$–$1d^1$) undergo more than two cleavages and may differentiate cilia of the apical tuft type. Neither second quartette micromeres (2a–2d) nor second generation macromeres (2A–2D) differentiate cilia. If an isolated trochoblast is permitted to cleave once, and the products separated, each product will later cleave but once before differentiating cilia. Such pairs of cells never cleave again, even though this ciliated unit may continue to swim about for two days or longer. In their capacity for self-differentiation the trochoblasts of *Nereis* therefore behave in essentially the same manner as those of *Patella*, described by Wilson (1904), and the isolation data are in agreement with the cell-lineage studies.

Blood clotting in Callinectes sapidus. James A. Donnellon.

The blood of the blue crab, *Callinectes sapidus*, contains the same cellular elements as those found in the crayfish, *i.e.*, amoebocytes, thigmocytes and explosive corpuscles.

Blood clotting involves both a cellular clot and a plasma clot. The plasma clotting takes place in two phases; first, a localized clotting around each explosive corpuscle, and secondly, a general clotting of the entire plasma.

Tissue extracts of the shell of the blue crab or of any of the internal structures bring about clotting both of the cells and of the plasma within a few minutes. Extracts of *Arbacia*, starfish, *Limulus*, mollusks and shrimp also hasten clotting. Isotonic solutions of various K salts and of $CaCl_2$, $BaCl_2$ and $SrCl_2$ produce the same effect.

Dilute solutions of fat solvents, hypertonic solutions, electrical stimulation, ultra-violet irradiation and mechanical stimulation also hasten clotting.

Isotonic $MgCl_2$, saturated $MgSO_4$ and a 10 per cent peptone solution will inhibit clotting indefinitely. Isotonic solutions of K-oxalate and Na citrate produce the same effect.

If the blood is oxalated and then filtered the cells can be completely removed from the plasma. If this cell-free plasma is then recalcified it will not clot. If cell extract is added to this plasma, however, clotting takes place within a few minutes. Extracts made of blood cells from *Arbacia*, starfish, *Limulus*, spider crab, *Venus mercenaria* and *Mactra* produce the same effect.

This effect of cell extracts on plasma clotting indicates that plasma clotting is initiated by a substance or substances liberated from the cells. In normal clotting several changes take place in the cells before the plasma clots; the granules in the amoebocytes break down, the thigmocytes cytolyze and the explosive corpuscles explode. These cell changes are initiated by the injury substance liberated when the animal is injured. Any substance which will preserve the cells will also prevent clotting, while tissue extracts and certain chemical and physical stimulants which break down the cells will cause clotting. Clotting also seems to be dependent upon the presence of Ca.

Effects of light and hormones upon the activity of young turtles, Chrysemys picta. Llewellyn Thomas Evans.

Between July 19 and August 18, 1940 each of five turtles was fed a total of 180 mg. of desiccated thyroid mixed with the food daily. Six turtles which received an average of 9 mg. of testosterone propionate [1] and seven untreated controls were fed identical food minus the thyroid. All 18 turtles weighed between 30 and 40 grams each.

Light-weight cages, suspended by light springs, to each of which were attached a stylus, recorded activity on 12-hour kymographs. Controls and thyroid-fed turtles were tested not only in normal daylight and dark but also in complete darkness for

[1] Kindly supplied by E. Oppenheimer, Ciba Pharmaceutical Products, Inc.

single periods of 24 hours. Those treated with testosterone propionate were tested only in normal daylight and dark.

By measuring only the periods of activity, but not the degree of intensity of activity, the following results, expressed in percentages, were obtained:

Under normal solar daylight conditions the controls were 20 per cent less active than those fed thyroid. In total darkness controls were 37 per cent less active than those fed thyroid. Controls were only 53 per cent as active in total darkness as in normal daylight, while turtles treated with thyroid were 81 per cent as active in total darkness as in normal daylight.

Those treated with testosterone propionate were 12 per cent more active, after treatment, under normal solar daylight.

Under normal conditions all turtles began activity soon after daylight and continued active until about noon; that was followed by relatively little activity, and after dark there was almost none at all. In total darkness, however, activity was sporadic; morning activity began at no fixed time and in a few instances the thyroid-fed animals were as active all night as in the morning. Controls, however, were quiet throughout the night and early morning hours. Activity of thyroid-fed animals was more intense during any given period of activity than that of controls.

Effects of testosterone propionate upon social dominance in young turtles, Chrysemys picta. Llewellyn Thomas Evans.

Where young turtles of different sizes were kept together, the largest dominated the group during feeding. Each of the others in turn dominated the next smaller. The dominating animal expressed its dominance toward another by climbing upon the carapace, snapping at or treading on the head, biting a foot, or approaching with open jaws. This behavior inhibited other animals so far as food-getting was concerned.

Groups were established as follows: I, II, III, IV, each with three animals of the same weight (within one gram); V and VI with three each, the largest being 12 to 17 per cent heavier than the smallest; VII to XVI with two each, the larger being between 25 per cent and 30 per cent heavier than the smaller. One turtle in each of I, II, III, IV and the smallest in V to XVI received, subcutaneously, testosterone propionate[1] dissolved in sesame oil. Dosage ranged from 3½ mg. for a turtle of nine grams weight to 19 mg. for a 49-gram turtle. The others in each group received equal amounts of sesame oil only.

More than 450 observations were recorded during daily feedings in July and August, 1940. Scores, based on objective signs of dominance listed above, were kept for each animal.

The turtles treated with hormone in all groups were more aggressive than the controls (injected with sesame oil only), except in groups VI, VII and XIV in which the larger controls maintained their dominance in varying degrees throughout the period of experiment.

All turtles treated with hormone showed markedly enlarged and swollen cloacal region.

The use of urethane as an indicator of "activity" metabolism in the sea urchin egg. Kenneth C. Fisher and Richard J. Henry.

The effect of various concentrations of ethyl urethane on the oxygen uptake of fertilized and unfertilized sea-urchin eggs has been determined along with the effect of the same inhibitor on cell division in the fertilized eggs. When the quantitative relation between inhibitor concentration and effect produced is examined (Fisher and Stern, *Biol. Bull.*, 1940), there is found a discontinuity in the relation suggesting

[1] Kindly supplied by E. Oppenheimer, Ciba Pharmaceutical Products, Inc.

that two parallel systems are summated to give the normal oxygen uptake. It is found further that the concentration of urethane just sufficient to completely block cell division is very nearly the concentration by which the more sensitive of the two respiratory systems is eliminated.

In the unfertilized sea-urchin egg, on the other hand, the oxygen consumption is uniformly sensitive to urethane, that is, the evidence obtained from the effect of urethane suggests a single respiratory system. The quantitative characteristics of this system with respect to inhibition by urethane are similar to the same characteristics for the less sensitive of the two respiratory systems indicated in the fertilized egg. It appears that fertilization initiates a second respiratory system (c.f., Ballentine, *J.C.C.P.*, 1940) which is added to the system already functioning in the unfertilized egg, and it may be through this second system that the energy required for division comes.

The inflation mechanism of Spheroides maculatus. Mordecai L. Gabriel.

The peculiar inflating habits of many of the plectognath fishes have long been familiar, but no satisfactory account of the inflating mechanism has yet been published.

In the present work, specimens of *Spheroides maculatus* were studied by observations on the intact fishes and following surgical removal of various structures. The action of particular muscles was observed through celluloid windows sewed into the ventral body wall, and by inductorium stimulation in anaesthetized animals.

Contrary to Thilo's belief (*Anat. Anz.* XVI: pp. 73–87), the post-clavicular apparatus plays no part in the puffing act. The post-clavicles are quite motionless during inflation, and fishes with both post-clavicles and the associated muscles removed showed no impairment of the inflating power.

The anatomy of the puffing mechanism is substantially as reported by Rosén (*Ark. f. Zool. Stockholm*, Bd. 7; No. 30). The essential part of the apparatus consists of the powerful muscles of the first branchiostegal ray, which depress a pad covering the ceratohyals, thus expanding the mouth cavity and drawing in air (or water). The elevation of the ceratohyals forces the air into the air-sac, which is a ventral diverticulum of the stomach, partially separated from it by a sphincter-like ring. This "sphincter" is not very contractile and remains open throughout the act of puffing. It does not serve to retain air in the air-sac, and inflation takes place perfectly well after its excision.

Air is retained in the diverticulum by a strong oesophageal sphincter and by the pylorus. The flap-like breathing valve in the mouth does little or nothing in this connection, and fishes in which this was lacerated or removed inflated rapidly. The opercular valves prevent leakage during the compression stroke, but the distended state of the air-sac can be maintained even when they are held open or removed.

Micromanipulative studies on the nuclear matrix of Chironomus salivary glands. E. A. Glancy.

Microdissection studies on freshly dissected salivary glands in Ringer's solution indicate that the chromosomes and nucleolus are imbedded in a central jelly of a weak consistency surrounded by a material which is more fluid. This is in accord with Duryee's findings on germinal vesicles of ovarian oöcytes in amphibians and fishes. The presence of protein in both jelly and the peripheral fluid is inferred from the precipitation produced when saturated ammonium sulfate, acids and heavy metals are introduced into the nucleus by a micropipette. Proteins may be further demonstrated by employing the oil drop retraction technique (Kopac, *Biol. Bull.*, **75**: 372, 1938). The evidence for a jelly-like matrix is as follows: (1) the nucleolus

and chromosomes move as a unit when one of these is pulled with a microneedle, (2) the nucleolus and chromosomes persist as a compact mass when the membrane of an isolated nucleus is torn, (3) centrally injected oil drops remain in place, whereas the natural tendency of oil to rise occurs when peripheral injections are made. In the nuclear fluid naturally occurring spherical particles (Nebennucleoli of Bauer) show an average displacement of one micron per second (Brownian movement). The consistency of this fluid is close to that of 40 per cent glycerol in water. This is shown by the similar magnitude of displacement by Brownian movement of carbon particles (less than one micron in diameter) either injected into the nuclear fluid or suspended in a 40 per cent glycerol solution. These observations suggest that the consistency of the nuclear fluid is approximately four times that of water. It may prove significant that there is a structural similarity between the atypical salivary gland nuclei which never divide and soon degenerate, and the completely functional nuclei of oöcytes which are to undergo many cycles of division.

The reversible nature of the potassium loss from erythrocytes during storage of blood at 2–5° C. John Harris.

When human blood is stored aseptically at 2–5°, potassium diffuses from the erythrocytes into the supernatant fluid (DeGowin, Harris, and Plass, *J. Amer. Med. Ass'n.*, **114**: 855 (1940)). The rate of loss is essentially that of a first-order reaction. Equilibrium is reached in 20–25 days. Thus the factor responsible for the shift is immediately operative.

The suggestion that the ammonia formed during preservation causes the cells to become permeable to potassium was not substantiated by experiments in which ammonium chloride was added to blood.

In the following experiments human blood was stored aseptically at 5° and then placed at 25° or 37°. The erythrocytes of a blood-glucose mixture (see aforementioned paper) stored at 5° lose 30 per cent of their potassium in 5–7 days and are apparently freely permeable to this ion. When this blood was placed at 37° no potassium diffused from the cell for periods of 6 to 24 hours. A migration of this ion from the plasma to the cells was usually seen, the cellular content increasing about 5 per cent. Cells from a 5-day-old citrate blood mixture, when placed at 37°, increased in potassium content by 15 per cent in 4 hours, thereby regaining a considerable proportion of the potassium they had lost during storage at 5°. After 4 hours the migration was again in the direction of cell to plasma. Such an accumulation was also seen at 25° although to a lesser extent. This reversibility of potassium permeation was completely absent when sodium fluoride (.02 M) was present. Under such conditions erythrocytes from a 7-day-old blood-glucose mixture when placed at 37° lost potassium very rapidly, about 15 per cent during the first hour and 60 per cent in 5 hours. Likewise sodium fluoride (.05 M) was found to induce a rapid loss of potassium from fresh human erythrocytes at 25°.

Rabbit erythrocytes have shown somewhat similar results although an actual increase in cellular content has not been observed.

It appears that the potassium content of the erythrocytes of human and possibly rabbit blood is maintained in some way by the cellular metabolism. When cells are stored at refrigerator temperatures the metabolism is lowered and the potassium diffuses from the cell, establishing a new equilibrium. When these cells are then placed at 37° the original state tends to be regained. The particular function affecting this equilibrium is inhibited by fluoride.

Studies on the life history of Anisoporus manteri sp. nov. (Trematoda: Allocreadiidae). Arne V. Hunninen and Raymond M. Cable.

The adult stage of *Anisoporus manteri* sp. nov. occurs in a number of marine fishes including the northern pipefish, *Syngnathus fuscus*, summer flounder, *Para-*

lichthys dentatus, killifish, *Fundulus heteroclitus*, and four-spined stickleback, *Apeltes quadracus*. It differs from the two described species of *Anisoporus* in the size of the body, dimensions of the eggs (0.062–.068 by 0.035–.04 mm.), shape of the ovary, and number of papillae on the ventral sucker (three anterior, two posterior). The cercaria is a modified cotylomicrocercous form, developing in sausage-shaped sporocysts in the branchial region and digestive gland of *Mitrella lunata*. It has a double-pointed stylet which is 0.011 mm. long, and three pairs of cephalic glands with a single lateral and two median ducts on each side. The tail is peculiar in that it does not form a hollow, sucker-like cup, but contains a mass of glands whose ducts converge to form a protrusible papilla, by means of which the larva may become firmly attached. The main ascending excretory tubules are ciliated and extend to the mid-acetabular level where each receives an anterior and posterior collecting tubule. The excretory formula is 2[(2 + 2) + (2 + 2)]; it remains unchanged during post-cercarial development. The cercariae penetrate and encyst in the hemocoele of marine amphipods, particularly *Carinogammarus mucronatus* and *Amphithoë longimana*. The metacercaria grows rapidly from less than 0.1 to over 0.7 mm. in cyst diameter. Adult structures become evident early in development and large metacercariae frequently contain eggs.

Histological studies on the problem of edema in haploid Triturus pyrrhogaster larvae. Cornelius T. Kaylor.

Edematous swelling of the body is the most common abnormality associated with haploid amphibian larvae obtained by experimental means. Occasionally it is also observed in diploid larvae which have developed from unoperated eggs.

In previous experiments on androgenesis in *Triturus pyrrhogaster*, 9 edematous haploid larvae and 1 edematous diploid control larva were preserved. The edema began in the living larvae at the time the circulatory system should have been functional. These larvae were sectioned and studied this summer. The edema was exhibited mostly in the head, pericardial and coelomic regions. The circulatory system of 8 haploid animals was not functional, as evidenced by the fact that blood cells were found only in the vitelline veins where blood cells are originally formed. Other vessels of the body were hypertrophied. In the ninth haploid larva only a few blood cells were present in the systemic circulation. The diploid larva also possessed a non-functional circulatory system. The heart was beating in all these larvae at the time of fixation.

The pronephros exhibited extreme hyperplasia: nephrostomes, tubules and collecting ducts were enlarged. The normal number of nephrostomes opened into the body cavity, the convoluted tubules connected with the collecting ducts and the latter were traced to an open cloaca. These observations suggest a functional compensation of the pronephric system for excessive amounts of body cavity fluids.

It would appear, then, that a non-functional circulatory system was responsible for the edematous condition of both haploid and diploid *I. pyrrhogaster* larvae.

Do carcinogenic compounds affect cell permeability? Balduin Lucké, Arthur K. Parpart and R. A. Ricca.

There are now available a number of chemical substances which on application to tissues induce cancer. The mechanism by which these substances affect cells is not known. However, since cancerous cells have been alleged to possess a degree of permeability different from that of normal cells, the possibility exists that a primary action of carcinogens upon cells is change of their surface properties and hence their permeability. If this be true for cells in general, it should be possible to detect this change in those cells in which permeability may accurately be meas-

ured. At present the only such cells are certain marine egg cells and mammalian erythrocytes.

In the present experiments the cells used were eggs of the sea urchin *Arbacia punctulata* and of the annelid *Chaetopterus pergamentaceus*, and erythrocytes of mouse and beef. The carcinogenic agents were choleic acid compounds of 20-methylcholanthrene, 1-2-5-6-dibenzanthracene, and 10-methylbenzanthracene. In addition, related non-carcinogenic compounds of phenanthrene, 1-2-benzanthracene, and acenapthene were tested.

The egg cells were exposed for a number of hours to saturated solutions of these substances in sea-water, after which their permeability to water was measured from curves of osmotic swelling in hypotonic solutions, using a diffraction method for following volume changes of cells (Lucké, B., Larrabee, M. G., and Hartline, H. K.: *J. Gen. Physiol.*, 1935, **19**: 1–17; Lucké, B., Hartline, H. K., and Ricca, R. A.: *J. Cell. & Comp. Physiol.*, 1939, **14**: 237–252). Unexposed cells were used as controls. The erythrocytes were shaken with water-saturated solutions of the compounds for 6 hours; their permeability was then determined for ethylene glycol, glycerol, erythritol, thiourea, the ammonium salts of acetic and butyric acids, and for chloride and sulfate ions. In addition, the lytic action of butyl alcohol on the cells was measured. In all cases, unexposed erythrocytes were likewise tested. A photoelectric method was used (Parpart, A. K.: *J. Cell. & Comp. Physiol.*, 1935, **7**: 153–162).

The results of these several determinations showed that neither the cells exposed to carcinogens nor those exposed to related non-carcinogenic compounds differed in their permeability from the control unexposed cells. This was true for both marine eggs and erythrocytes.

The development of the skull in the non-aquatic larva of the tree-toad Eleutherodactylus nubicola. W. Gardner Lynn.

The Jamaican tree-toad, *Eleutherodactylus nubicola*, is one of the few anurans which deposits its eggs on land and has no aquatic tadpole stage in its life-history. The animal never possesses the typical tadpole suctorial mouth and larval teeth, and neither external nor internal gills are every present. The development of the cranium and branchial apparatus is modified in relation to these features. A complete series of developmental stages has been sectioned and studied and wax reconstructions of the skulls of five critical stages have been made. Post-embryonic stages have been studied by the use of cleared specimens.

The chondrocranium differs markedly from that of *Rana* in the absence of the supra- and infrarostral cartilages and in the late appearance of the quadratocranial commissure connecting the quadrate with the trabecula. These structures are directly concerned with the support of the suctorial mouth in ordinary tadpoles. The synotic tectum and the tectum transversum are formed relatively late and closure of the foramen prooticum by growth of the taenia marginalis does not occur until after the orbital cartilages have enclosed the optic foramen.

The hyobranchial skeleton is represented in early stages by four paired cartilages connected to a broad central plate. Its later development is a direct transformation into the definitive, typical frog hyoid which is fully formed several days before hatching. The fourth ceratobranchial is lacking.

The osteocranium ossifies in a succession which differs from that found in *Rana*. The most noteworthy feature is the early appearance of the angular, squamosal and dentary which in *Rana* do not develop until the time of metamorphosis. Aside from these, most of the bones which are formed during or after metamorphosis in *Rana* are not present in *Eleutherodactylus* until some time after hatching even though, at hatching, this animal already possesses the adult body form.

The embryonic origin and development of the pharyngeal derivatives in Eleutherodactylus nubicola. W. Gardner Lynn.

Since the Jamaican tree-toad, *Eleutherodactylus*, possesses neither external nor internal gills and has no open gill-slits during its embryonic history, it affords an excellent opportunity for a detailed study of the origin of the various pharyngeal derivatives. Sections of a large series of embryos have been studied directly and by means of wax-plate reconstructions.

As in *Rana*, the first visceral pouch, the hyomandibular, is never well-defined but the rod of cells which represents the anlage of the middle ear cavity at late stages probably marks its position. Three branchial pouches are formed. The thymus body is derived exclusively from the dorsal wall of branchial pouch I and there is no evidence of the existence of the transitory thymus body which Maurer found as a derivative of the hyomandibular pouch in *Rana*. Branchial pouches II and III give rise to paired epithelioid bodies which originate from the ventral medial walls of the pouches and come into close relations with the systemic and pulmocutaneous trunks.

The postbranchial body is paired as in *Rana*, not unilateral as in *Amblystoma*. It is truly "postbranchial" in that it arises from the pharyngeal wall behind the most posterior visceral pouch.

The carotid glands appear quite late and, contrary to Maurer's findings for *Rana*, seem to be differentiated exclusively from the walls of the carotid arch.

A search for the "Kiemenreste" of Maurer has failed to reveal such structures and, in view of the absence of gills in this frog, this would seem to support Maurer's original conclusions as to their origin rather than Norris' later claim that they are of extra-branchial derivation.

The thyroid develops from the pharyngeal floor in the usual way and, as the author has shown in a previous study, becomes active very early; a fact which is doubtless correlated with the precocious course of bodily differentiation in this anuran.

Preliminary report on effect of indole acetic acid on growth of Chlamydomonas. Sister Maria Laurence Maher.

Reports on effect of auxin on growth of algae made by Brannon and Bartsch, Leonian and Lilly, Robertson Pratt and Yin indicate that different culture media may play a part in the different results obtained.

In these experiments pond water, glass-distilled water and soil solution were media used. These were made up with concentrations of 2½-5-10-15-20-30-40 and 50 p.p.m. of indole acetic acid with each media alone and also with the media containing .1 per cent dextrose.

Ten-cc. amounts of solutions were placed in cotton-stoppered Pyrex tubes and inoculated with 1 cc. of solution containing cells that were previously taken from agar slants and placed in solution so that most of the cells were in a motile condition. pH was adjusted at the beginning of each experiment and when counts were made, to 7 by use of sodium hydroxide and acetic acid.

Rate of growth was determined by increase in number of cells shown by haemocytometer counts.

Higher concentrations in all cases showed definite stimulation. In pond water, the peak of growth was reached in seven days with a concentration of 15 p.p.m. of auxin giving best results.

In glass-distilled water, the peak of growth was reached in two weeks with concentrations of 30 and 40 p.p.m. giving typical growth curves. Further work on factors causing variation in different experiments is needed. Rate of change of pH and type of multiplication appear to play an important part in these variations.

Rapidity of growth in soil solutions makes counts difficult because of the tendency

of the rapidly growing cells to clump together. A number of cultures were rejected because of bacterial contamination and considerably more work with this culture is needed before conclusions may be drawn.

Further studies on the metabolism of cell fragments. Herbert Shapiro.[1]

From earlier work on the oxidative activity of the light and heavy fragments of the egg of the sea urchin *Arbacia punctulata*, obtained by centrifuging, the first measurements on their oxygen uptake, secured with Warburg manometers, were reported (Shapiro, H., *Jour. Cell. Comp. Physiol.*, **6**: 101, 1935). These experiments have been repeated and extended in order to study the effect of potassium cyanide on both unfertilized and fertilized "halves." Microrespirometers of the Fenn-Winterstein type were used, modified, after Schmitt, by the introduction of a bridge stopcock. Preliminary investigation shows that the light unfertilized halves are relatively little affected by KCN, whereas the light fertilized halves are markedly affected by this agent, their oxygen uptake being considerably reduced. The temperature at which the experiments are carried out may determine the inhibitory effectiveness. Cyanide also completely inhibits the cleavage of the fertilized light fragments. The unfertilized heavy fragments were cyanide-sensitive, and the oxygen uptake by fertilized heavy halves was likewise depressed, in most of the experiments, in the presence of cyanide.

Responses to acetylcholine and cholinesterase content of Cerebratulus. Carl C. Smith, Blanche Jackson, and C. Ladd Prosser.

Cholinergic systems have been indicated in the annelids (Bacq and Copée, *Arch. Int. Phys.*, **45**: 310, 1937) but in no lower groups of animals. Preparations of the dorsal longitudinal muscle of the flatworm, *Cerebratulus lacteus*, were suspended in sea water and the effect of acetylcholine examined. No response was obtained in a concentration of 10^{-4} acetylcholine hydrochloride without eserine. After eserinization, contractions were obtained in concentrations of 5×10^{-6} acetylcholine. Increase of the concentration of acetylcholine after eserine increased the rate and height of the contraction. This sensitization by eserine led us to examine the cholinesterase content by titration and manometric methods. Three manometric determinations gave an average CO_2 liberation of 214.5 cu.mm./30 min./50 mg. tissue, wet weight. This value was corrected for non-enzymatic hydrolysis. The esterase content of *Cerebratulus* is much higher than has been reported for other invertebrate body muscles; this esterase content may explain the high degree of sensitization to acetylcholine by eserine. This evidence is indicative of a cholinergic system in the flatworms.

Response of the heart of the compound ascidian, Perophora viridis, to pilocarpine, atropine and nicotine. A. J. Waterman.

Pilocarpine (1–2000 to 1–10,000) may cause immediate increase in the length of the abvisceral phases for 10–40 minutes after exposure is begun, but this increase is less than that provoked by mecholyl, acetylcholine and especially adrenalin. Increasing depressive effects soon ensue accompanied by great irregularity in number of beats. In contrast, the normal advisceral rhythm may be at first maintained but depression appears earlier than for the abvisceral phase and soon the advisceral count may be reduced to 1–4 in number. Except for these short interruptions, the abvisceral phase practically becomes continuous. Rate in both phases becomes

[1] Aided in part by a grant from the Penrose Fund of the American Philosophical Society. Part of the apparatus used was made available by the Beadle fund of Vassar College.

reduced and irregular, the interval between reversals is increased, and long pauses become increasingly common. One to 500 reduces heart action to spasmodic twitching at the ends in 12–15 minutes. There appears to be no hindrance to the immediate penetration of all drugs tested; small animals are less sensitive than large ones; the advisceral phase is always the first to show depressive effects; considerable variation in response is shown by different animals; and even inactive hearts recover in sea water.

Atropine acts as a depressant in proportion to the concentration. One to 100,000 is relatively non-depressive, while 1–50,000 stops most hearts, except for spasmodic twitching at the ends, after 4–7 hours. Preliminary exposure for 20–30 minutes to non- or slightly depressive concentrations, (1) inhibits the initial stimulatory action of pilocarpine although the subsequent depressive effects, which may be intensified, are frequently characteristic of pilocarpine, and (2) sensitizes the heart to depressive action of pilocarpine. Combining non-depressive concentrations of both drugs quickly provokes and intensifies depression and irregularity in both phases.

Nicotine similarly depresses heart action. One to 500,000 was without effect during one hour but later caused marked reduction, while 1–50,000 acted immediately.